



## The effect of age, sex and postmortem aging on meat quality traits and biochemical profile of different muscles from Brangus cattle



Julieta Fernández Madero <sup>a,b \*</sup>

Laura Pouzo <sup>a,c</sup>

Darío Pighín <sup>d,e</sup>

Jorge Alejandro Navarro <sup>f</sup>

Fernando Ailán <sup>g</sup>

César Federico Guzmán <sup>h</sup>

Enrique Paván <sup>a,c</sup>

<sup>a</sup> Universidad Nacional de Mar del Plata. Facultad de Ciencias Agrarias. Balcarce, Buenos Aires, (7620), Argentina.

<sup>b</sup> Universidad Católica de Salta. Facultad Ciencias Agrarias y Veterinarias. Salta, Argentina.

<sup>c</sup> Instituto Nacional de Tecnología Agropecuarias, EEA Balcarce, Balcarce, Argentina.

<sup>d</sup> Instituto Nacional de Tecnología Agropecuarias. Instituto Tecnología de Alimentos - CNIA - Castelar. Buenos Aires, Argentina.

<sup>e</sup> Consejo Nacional de Investigaciones Científicas y Técnicas - CONICET. Argentina.

<sup>f</sup> Universidad Nacional de Salta. Salta, Argentina.

<sup>g</sup> Universidad Nacional de Tucumán. Tucumán, Argentina.

<sup>h</sup> Instituto Nacional de Tecnología Agropecuarias, EEA Cuenca del Salado, Argentina.

\* Corresponding author: [jfernandez@ucasal.edu.ar](mailto:jfernandez@ucasal.edu.ar)

**Abstract:**

Carcass quality traits and the *Longissimus thoracis* (LT) and *semitendinosus* (ST) muscles, aged for 2 or 14 d, from sixty castrated (CM) and non-castrated (NCM) Brangus males, slaughtered at 16 (M16) or 20 (M20) mo of age (391 and 434 kg live weight; 3.81 and 4.25 mm backfat thickness respectively), were evaluated. The carcasses of castrated and younger animals weighed less than those of non-castrated and older ones ( $P<0.001$ ). Castration produced more subcutaneous fat and lower rib eye areas ( $P<0.05$ ). Temperature and pH decline was faster in younger animals, and final pH was lower in castrated ( $P<0.05$ ). While the Warner Bratzler Shear Force (WBSF) of LT was 9 % lower in castrated, and 7 % higher in younger animals ( $P<0.05$ ); it decreased with a longer aging period ( $P<0.001$ ). The WBSF of LT was positively associated with total collagen content ( $r=0.54$ ;  $P<0.01$ ) and negatively with myofibrillary fragmentation index ( $r=-0.39$ ;  $P<0.05$ ). The WBSF of ST was not affected by animal castration or slaughter age ( $P>0.05$ ), but decreased with a longer aging period ( $P<0.001$ ), and was positively associated with total collagen content ( $r=0.61$ ;  $P<0.05$ ). Both muscles from castrated slaughtered at younger ages had the higher  $L^*$  values. It is concluded that castration and age at slaughter on Brangus males produced differences on WBSF values only on LT muscle where collagen is not the main determinant of shear force.

**Keywords:** Beef, Brangus, Collagen content, Color, Myofibrillar fragmentation index, Shear force, Temperature decline.

Received: 22/07/2023

Accepted: 28/09/2023

## Introduction

Non-castrated males are an interesting alternative for beef producers to obtain leaner or heavier carcasses<sup>(1,2)</sup>. Testosterone is the main hormone produced in uncastrated males. Among its functions include the development of male organs, secondary sexual characteristics, and promoter of muscle development. This anabolic property directly influences the daily weight gain and feed efficiency, producing a carcass with a higher yield of retail product with less fat and more red meat than castrates<sup>(3)</sup>. Differences in favor of bulls are generally more pronounced with increasing slaughter weight<sup>(1)</sup>. However, lean carcasses with low fat thicknesses could result in a fast temperature decline, leading to tougher cuts<sup>(4)</sup>. The lower tenderness of meat from non-castrated than from castrated males was associated with its higher content of connective tissue and lower endogenous protease activity responsible for *postmortem* tenderization<sup>(2,5)</sup>. On the other hand, as animals get older, meat

collagen solubility decreases<sup>(6)</sup> and myoglobin concentration increases<sup>(7)</sup>. Therefore, tougher and darker meat cuts could be expected with increasing animal age<sup>(1)</sup>.

It has been proposed that the effect of castration and animal age at slaughter on meat color and tenderness varies with muscle type<sup>(5,8)</sup>. In addition, the response to *postmortem* aging also varies with type of muscle<sup>(5,9)</sup>. Rodriguez *et al*<sup>(5)</sup> found no effect of castration on Warner Bratzler Shear Force (WBSF) in muscles with high amount of connective tissue (*Psoas major*, *semitendinosus* (ST)); however, they observed effect of castration in WBSF in the *Longissimus* muscle. Tenderness would be determined mainly by the high collagen content and solubility in *semitendinosus* muscle, and by higher *postmortem* proteolysis activity in muscles like *Longissimus thoracis* (LT)<sup>(9)</sup>. Therefore, the effects of castration and age at slaughter on meat color and tenderness varies with the type of muscle considered<sup>(5,10)</sup> as well as with the *postmortem* aging period<sup>(11)</sup>.

Only a few studies have evaluated the effects of castration or slaughter age on animal performance and carcass characteristics of Brangus cattle<sup>(12,13)</sup>, but none of them evaluated the interaction of these effects have on the meat quality of different muscles. Therefore, the aim of the present study was to evaluate the effect of castration and slaughter age of Brangus males on carcass quality and biochemical profile of two muscles of different characteristics, the LT and the ST.

## Material and methods

The trial was carried out following the Good Manufacturing Practices and welfare standards for animal handling recommended by the Argentine National Institute for Agricultural Technology (INTA). The trial was approved by the institutional ethical and technical committee of the Catholic University of Salta (RR N° 694/12). The study was conducted in General Güemes, Salta province, Argentina (24°42'40.8"S, 64°57'48.8"W, 670 m altitude).

### Animals and treatments

Sixty (60) Brangus calves of similar age (7 mo) and weight ( $178 \pm 13$  kg) were randomly selected from the same cow-calf herd and assigned to one of the four treatment combinations defined by the sex category (CM, castrated males, and NCM, non-castrated males) and age at slaughter (M16, males slaughtered at 16 mo of age, and M20, males slaughtered at 20 mo of age). Each combination involved 15 animals. At 7 mo of age those animals assigned to CM were surgically castrated. Animals were reared on a paddock of alfalfa and supplemented with a mix of whole corn grain (25 % DM), whole plant sorghum silage (72.5 % DM) and vitamin-mineral core with monensin (2.5% DM) fed at 1.5 % of live weight until they were enclosed in a pen. The rearing period was 3 mo for M16 and 7 mo for 20. For M16 the live

weight of entry to the pens was  $192 \pm 3$  kg and for M20  $293 \pm 9$  kg. During enclosing the concentrate diet consisted of cracked corn grain (57.25 % DM), whole plant corn silage (26 % DM), sunflower or cotton pellets (13.5 % DM), granulated urea (0.75 % DM) and a mineral vitamin supplement with monensin (2.5 % DM). Live weight was determined every 28 d. The average daily gain and feed efficiency observed during the enclosing period was  $0.96 \pm 0.11$  kg/d and  $8.5 \pm 0.9$  kg/kg for the CM and  $1.11 \pm 0.12$  kg/d and  $7.6 \pm 1.1$  kg/kg for the NCM, irrespective of the slaughter age.

### **Carcass measurement and sample collection**

The day before slaughter, animals were weighed individually to record their full live weight (LW) and shipped to the slaughterhouse located 350 km from the experimental farm (driving time of 5 h), where they were kept in lairage for 12 h prior to slaughter, with free access to water and feed withdrawal.

Carcasses were electrically stimulated (21 V 0.25 A) at two independent stimulation times of 20 and 30 sec); then, hot carcass weight (HCW) was recorded. Dressing percentage was calculated by dividing the HCW by the pre-shipment full LW of the animal  $\times 100$ . The muscle pH and temperature were recorded between 12<sup>th</sup> and 13<sup>th</sup> ribs *Longissimus thoracis et lumborum* of the left carcass side at 2, 5, 8, 14 and 26 h *postmortem* using a Testo 205 pHmeter. To estimate the decline of pH and temperature, and carcass cooling rates, the pH/temperature window concept implemented in Meat Standards Australia (MSA) was used. This concept includes the measurement of temperature when the pH value = 6 (Temp@pH6) and measurement of pH when the temperature value = 12 °C (pH@Temp12).

After 48 h of chilling, the ultimate pH (pHu) was measured at the 12<sup>th</sup> rib of the left side of the carcasses. Back fat thickness (BFT) was measured at between 12<sup>th</sup> and 13<sup>th</sup> ribs using a digital caliper (Starrett 125). The LT rib eye area (REA) was recorded on the 12<sup>th</sup> rib, and then analyzed by Image APS-Asses Ink software (University of Manitoba, Winnipeg, Manitoba, Canada, 2002). The LT and ST muscles were sampled from the left side of the carcasses. The 8 - 12<sup>th</sup> rib section was obtained from the left side of each carcass by cutting perpendicularly to the long axis of the LT muscle in the joints of the 7<sup>th</sup>–8<sup>th</sup> and 12<sup>th</sup>–13<sup>th</sup> dorsal ribs. The whole ST muscle from the left side of each carcass was also obtained during carcass fabrication at 48 h *postmortem*.

### **Sample preparation and *postmortem* treatments**

Four 1.5-cm and two 2.5-cm thick steaks were obtained from caudal to cranial from each muscle sample. The 1.5-cm thick steaks were immediately vacuum-packaged and stored at  $-20$  °C for subsequent determination of sarcomere length (SL), total lipid content,

myofibrillary fragmentation index (MFI), glycolytic potential and total and soluble collagen content. The 2.5-cm thick steaks were randomly assigned to one of two aging periods (2 and 14 d) in vacuum at 4 °C. After the aging period, meat samples were stored at -20 °C until WBSF and color evaluation.

## **Meat quality evaluation**

### **Color**

Instrumental color measurements were taken after 30 min of blooming. Readings were performed with a Minolta CR-310 (Minolta Corp, Ramsey, N.J.) using a 50-mm diameter measuring area, a 10° standard observer and a D65 illuminant. The system used was the CIE Lab, which provides three color components: L\* (lightness, 0= black, 100= white), a\* (red index, -a\*= green, +a\*= red) and b\* (yellow index, -b= blue, +b= yellow). Values were recorded in three locations of the exposed area to obtain a representative reading.

### **Total lipid content**

Total lipid content (g of lipids/100 g of fresh tissue) was determined using an automatic extraction system (Ankom xt10, Ankon, Macedon NY, USA) and petroleum ether as solvent<sup>(14)</sup>.

### **Warner Bratzler Shear Force**

The WBSF analysis was conducted following the guidelines of AMSA, 1995<sup>(15)</sup>. Steaks were thawed at 4 °C for 12 h and cooked on preheated open-heart electric grill (Farberware, Bronx, New York) at an internal temperature of 71 °C. Steaks were cooled at 4 °C for 1 h; then six 1.27-cm diameter cores were removed from each steak parallel to the muscle fiber orientation. Meat cores were cut perpendicularly to the long axis of muscle sample using a WBSF testing machine (G-R Manufacturing, Manhattan, KS, US) equipped with a digital dynamometer.

### **Total and soluble collagen content**

Total collagen content was estimated by determining hydroxyproline using the procedure described by Bergman and Loxley<sup>(16)</sup>. Insoluble collagen content was determined using a procedure adapted from Hill<sup>(17)</sup>. Soluble content was estimated as the difference between total and insoluble collagen content.

### **Sarcomere length**

Three grams of muscle tissue was homogenized in 20 ml of solution 0.25 M sucrose at 4 °C for 15 sec with a disperser (CAT x 120, Germany)<sup>(18)</sup>. Sarcomere length was determined using a diffraction laser (CVI Melles Gliot. Series 7822 FH-1)<sup>(18)</sup>.

**Glycolytic potential**

Glycolytic potential was calculated from muscle glycogen and lactate concentration, where  $GP = 2 (\text{glucose 6-phosphate} + \text{Glycogen} + \text{glucose}) + \text{lactate}^{(19)}$ .

**Glycogen content**

Muscle glycogen content was extracted from muscles by acid hydrolysis<sup>(20)</sup>. Briefly, about 500 mg of muscle samples were homogenized (Ultraturrax, Fisher Scientific) for 30 sec in 5 mL 2 N HCl, and then, submitted to hydrolysis at  $100 \pm 1$  °C for 2 h. Glucose released was measured spectrophotometrically (505 nm; Spectrophotometer Thermo Fisher Scientific USA) in the neutralized homogenates (2 N NaOH) with the GOD/ POD Trinder Color test (GT Wiener Lab, Rosario, Argentina). Available glycogen content was expressed as mmol of glucose per gram of wet tissue. The quantified glucose included free glucose and glucose from glycogen hydrolysis<sup>(20)</sup>.

**Lactate content**

Muscle lactate was determined spectrophotometrically (550 nm; spectrophotometer-Thermo Fisher Scientific, USA), following the procedure described by Neath *et al*<sup>(21)</sup> and using a commercial kit (Randox kit LAC; Randox Laboratories Ltd, Crumlin, Co. Antrim, UK).

**Myofibrillary fragmentation index**

Protein concentration was determined by calculating MFI according to the protocol described by Hopkins *et al*<sup>(22)</sup>, using a microplate Spectrophotometer equipped with an Epoch-type reader (Biotek, USA).

## Statistical analysis

Statistical analysis was performed using the mixed procedure of the Statistical Analysis System R (Version 3.6.1). Data were analyzed separately for each muscle (LT, ST). Color and WBSF data were tested as a split-plot design, where effects of sex and age at slaughter were considered in the main plot and *postmortem* aging period effect was considered as a sub-plot. All possible interactions between individual factors were computed in the model. Data of variables in which the effect of the aging period was not included (pH and temperature decline, animal live weight and carcass characteristics, sarcomere length, intramuscular fat (IMF), total and soluble collagen, glycogen, MFI) were analyzed under a completely randomized design with a 2 x 2 factorial arrangement (two categories and two slaughter ages). For the variables of carcass characteristics (pH and temperature decline, dressing percentage, ribeye area and backfat thickness) the LW was considered as a covariate. Least square means were computed for main and interactive effects and separated statistically using F-protected ( $P < 0.05$ ) t-tests. To evaluate the degree of association between the

different physicochemical variables that explain color and tenderness, Pearson correlations were used ( $P \leq 0.05$ ).

## Results

### General characteristics

Table 1 shows the effect of age and category on LW and carcass characteristics. A significant interaction between sex category and slaughter age (S x SA) was observed for LW ( $P < 0.001$ ). At older age (M20), LW increased by about 4 % in CM and 9 % in NCM. Hot carcass weight was lower in CM than in NCM, and in M16 than in M20 ( $P < 0.001$ ). Regardless of slaughter age, BFT was 30 % higher ( $P < 0.01$ ) in CM than in NCM, and REA was 11% lower ( $P < 0.001$ ). The ultimate pH was lower in CM than in NCM ( $P < 0.05$ ; 5.46 and 5.53, respectively).

The pH and temperature decline of the LT muscle was influenced by the interaction between slaughter age and time of measurement (S x TM;  $P < 0.001$ ; Table 2). Temperature of M16 and M20 decreased as the *postmortem* time of measurement progressed, but at different speeds. Although the initial and final temperatures (2 and 26 h *postmortem*) of LT were similar for M16 and M20, the LT temperatures at 5, 8 and 14 h *postmortem* were lower for M16 than for M20. In addition, muscle temperature was higher in CM than in NCM, irrespective of the *postmortem* time ( $P < 0.001$ ; 12.06 and 11.13 °C, respectively). Muscle pH was 2.2 % higher in M16 than in M20 only at 2 h *postmortem*, with no differences being observed in the remaining *postmortem* measurement times ( $P > 0.05$ ).

**Table 1:** Effect of sex category and slaughter age on live weight and carcass characteristics of Brangus cattle

	M16		M20		SEM	Significance		
	CM	NCM	CM	NCM		S	SA	S x SA
<b>Animal live weight and Carcass characteristics</b>								
Live weight, kg	393.84 <sup>c</sup>	404.70 <sup>b</sup>	410.76 <sup>b</sup>	443.97 <sup>a</sup>	4.07	***	***	***
Hot Carcass weight, kg	218.67 <sup>c</sup>	228.33 <sup>b</sup>	235.53 <sup>b</sup>	252.93 <sup>a</sup>	3.19	***	***	ns
Dressing percentage (HCW/LW x 100)	56.32	56.66	57.14	56.45	0.54	ns	ns	ns
Backfat thickness, mm	4.55 <sup>a</sup>	3.07 <sup>b</sup>	4.55 <sup>a</sup>	3.95 <sup>b</sup>	0.50	**	ns	ns
Ribeye area, cm <sup>2</sup>	57.30 <sup>a</sup>	63.29 <sup>b</sup>	59.16 <sup>a</sup>	67.50 <sup>b</sup>	1.70	**	ns	ns
Temp@pH6	17.51	16.73	19.58	19.59	1.46	ns	ns	ns
pH@Temp12	5.74	5.81	5.75	5.80	0.07	ns	ns	ns
pH <sub>u</sub>	5.42 <sup>a</sup>	5.57 <sup>b</sup>	5.45 <sup>a</sup>	5.61 <sup>b</sup>	0.02	*	ns	ns

M16= males slaughtered at 16 mo of age; M20= males slaughtered at 20 mo of age; NCM= non-castrated males; CM= castrated males; SEM= standard error of the mean; S= sex category; SA= slaughter age; S x SA= interaction between sex category and slaughter age; Temp@pH6= muscle temperature when the pH is 6; pH@Temp12= pH value when muscle temperature is 12 °C; pH<sub>u</sub>= ultimate pH at 24 h *postmortem*;

<sup>abc</sup> LS-means with different superscripts within a row are different ( $P < 0.05$ ). \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ; ns=  $P > 0.1$

**Table 2:** Evolution of temperature and pH measured in the *Longissimus thoracis* muscle during the first 26 h *postmortem* in Brangus cattle non-castrated and castrated males slaughtered at 16 months or at 20 months of age

Slaughter age	Sex category	M16		M20		SEM	Significance
		CM	NCM	CM	NCM		
pH	TM						
	2	6.28 <sup>a</sup>	6.35 <sup>a</sup>	6.18 <sup>b</sup>	6.18 <sup>b</sup>	0.02	SA **: TM ***; SA x TM: ***
	5	5.81	5.84	5.97	5.91		
	8	5.69	5.67	5.78	5.72		
	14	5.56	5.54	5.62	5.65		
	26	5.43	5.45	5.55	5.61		
Temperature	2	23.23 <sup>A</sup>	22.43 <sup>B</sup>	23.65 <sup>A</sup>	23.10 <sup>B</sup>	0.12	S: ***; SA: ***; TM: ***; SA x TM: **
	5	15.38 <sup>Aa</sup>	14.15 <sup>Ba</sup>	17.49 <sup>Ab</sup>	16.39 <sup>Bb</sup>		
	8	8.96 <sup>Aa</sup>	6.88 <sup>Ba</sup>	13.86 <sup>Ab</sup>	12.24 <sup>Bb</sup>		
	14	3.97 <sup>Aa</sup>	2.43 <sup>Ba</sup>	8.25 <sup>Ab</sup>	7.45 <sup>Bb</sup>		
	26	3.74 <sup>A</sup>	3.69 <sup>B</sup>	2.79 <sup>A</sup>	2.59 <sup>B</sup>		

M16= males slaughtered at 16 mo; M20= males slaughtered at 20 mo; NCM= non-castrated males; CM= castrated males; TM= time of measurement; SEM= standard error of the mean; S= sex category; SA= slaughter age. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ . ns=  $P > 0.05$  not significant effects ( $P > 0.1$ ) are not described.

Different capital letters indicate differences between S and SA.

Different letters indicate differences between SA and PA

**Warner Bratzler Shear Force, glycolysis variables, and meat color**

The WBSF of the LT muscle was affected by the two main effects evaluated ( $P < 0.05$ ), but by none of their interactions ( $P > 0.05$ ; Table 3). The WBSF was 9 % lower in CM than in NCM, 7 % higher in M16 than in M20, and 36 % higher with 2 d than 14 d of *postmortem* aging. In contrast, the WBSF of ST was affected only by the aging period, decreasing by 12 % from 2 to 14 d.

**Table 3:** Effect of sex category and slaughter age on *Longissimus thoracis* (LT) and *semitendinosus* (ST) muscle characteristics (color and WBSF) of Brangus cattle

Slaughter age		M16				M20				SEM	Significance
		CM		NCM		CM		NCM			
Sex category		2 days		14 days		2 days		14 days			
PA		2 days		14 days		2 days		14 days			
LT	WBSF (N)	42.43 <sup>wx</sup>	30.91 <sup>yz</sup>	44.32 <sup>w</sup>	32.67 <sup>yz</sup>	37.59 <sup>xy</sup>	27.48 <sup>z</sup>	43.40 <sup>wx</sup>	31.91 <sup>yz</sup>	1.00	S*, SA*, PA***
	Color										
	L*	43.45 <sup>Aa</sup>	42.68 <sup>Aab</sup>	42.25 <sup>ABa</sup>	41.52 <sup>ABab</sup>	40.52 <sup>Bb</sup>	41.95 <sup>Bab</sup>	41.73 <sup>ABb</sup>	42.24 <sup>ABab</sup>	0.19	SA*, S x SA*, SA x PA*
	a*	22.52	21.85	21.72	22.35	21.58	22.82	21.22	21.91	0.14	
	b*	15.71 <sup>a</sup>	14.73 <sup>ab</sup>	14.86 <sup>a</sup>	14.73 <sup>ab</sup>	14.25 <sup>b</sup>	15.09 <sup>ab</sup>	14.36 <sup>b</sup>	14.45 <sup>ab</sup>	0.10	SA*, SA x PA*
ST	WBSF (N)	42.15 <sup>wx</sup>	36.99 <sup>y</sup>	44.75 <sup>w</sup>	38.17 <sup>xy</sup>	43.43 <sup>w</sup>	38.42 <sup>xy</sup>	43.06 <sup>w</sup>	40.76 <sup>wxy</sup>	1.06	PA***
	Color										
	L*	49.17 <sup>A</sup>	45.29 <sup>A</sup>	47.87 <sup>A</sup>	45.03 <sup>A</sup>	46.48 <sup>B</sup>	42.20 <sup>B</sup>	48.53 <sup>A</sup>	43.56 <sup>A</sup>	0.35	SA**, PA***, S x SA*
	a*	14.17 <sup>c</sup>	18.30 <sup>a</sup>	14.06 <sup>c</sup>	18.35 <sup>a</sup>	18.05 <sup>a</sup>	17.67 <sup>c</sup>	15.41 <sup>a</sup>	17.24 <sup>c</sup>	0.28	PA***, SA x PA***
	b*	20.29 <sup>b</sup>	19.53 <sup>b</sup>	20.03 <sup>b</sup>	19.49 <sup>b</sup>	22.19 <sup>a</sup>	18.33 <sup>c</sup>	21.16 <sup>a</sup>	18.43 <sup>c</sup>	0.21	PA***, SA x PA***

M16= males slaughtered at 16 mo; M20= males slaughtered at 20 mo; NCM= non-castrated males; CM= castrated males; PA= *postmortem* aging period; SEM= standard error of the mean; WBSF= Warner Bratzler Shear Force; L\* (lightness), a\* (red index) and b\* (yellow index); S= sex category; SA= slaughter age. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ; ns=  $P > 0.05$ .

not significant effects ( $P > 0.1$ ) are not described.

<sup>wxy</sup> LS-means with different superscripts within a row are statistically different ( $P < 0.05$ ).

Different capital letters indicate differences between S and SA.

Different letters indicate differences between SA and PA

Neither the LT muscle intramuscular fat (IMF) nor the sarcomere length (SL) or the myofibrillar fragmentation index (MFI) was affected by the treatments ( $P>0.05$ , Table 4). The LT total collagen (TC) content was lower ( $P<0.01$ ) but the proportion of soluble collagen (SC) content was higher ( $P<0.001$ ) in CM than in NCM. In the LT muscle, the proportion of SC was reduced (39 %) with increasing slaughter age ( $P<0.001$ ). The LT muscle glycogen concentration was 5 % higher in the M20 than in M16 ( $P<0.05$ ). The WBSF of LT was positively associated with total collagen content ( $r= 0.54$ ;  $P<0.01$ ) and negatively with myofibrillary fragmentation index ( $r= -0.39$ ;  $P<0.05$ ).

As in the LT, the TC content of the ST muscle was lower ( $P<0.05$ ) in CM than in NCM. The ST muscle from the CM had greater sarcomere length than that from the NCM ( $P<0.001$ ). The IMF of the ST muscle was higher in M16 than in M20 ( $P<0.05$ ), but no effects were observed between sex categories ( $P>0.05$ ). The WBSF of ST was positively associated with total collagen content ( $r= 0.61$ ;  $P<0.05$ ).

The lightness ( $L^*$ ) of the LT muscle was affected ( $P<0.05$ ; Table 2) by the S x SA interaction or by slaughter age x *postmortem* aging period (SA x PA) interaction. The highest  $L^*$  in LT was observed in CM-M16, and the lowest one in CM-M20, with the  $L^*$  of the NCM being intermediate and similar between M16 and M20. In addition, the  $L^*$  and  $b^*$  of the LT were higher for the M16 steaks aged for 2 d than for those from M20 aged also for 2 d, whereas steaks from M16 and M20 aged for 14 d had intermediate values, with no differences from those of M20 aged for 2 d ( $P<0.05$ ; Table 3).

In contrast, the  $L^*$  of the ST muscles was lower in CM-M20 ( $P<0.05$ ). In turn, the  $a^*$  and  $b^*$  of the ST muscle were affected by the interaction between slaughter age and aging period. The  $a^*$  of the ST muscle was higher for M16 aged for 14 d than for M20 aged for 2 d, being intermediate for M20 aged for 14 d, whereas the ST muscle from M16 aged for 2 d had the lowest  $a^*$  ( $P<0.001$ , Table 2). The  $b^*$  of the ST muscle was highest for M20 meat aged for 2 d and lowest for M20 meat aged for 14 d ( $P<0.001$ ), being intermediate for M16 meat aged 2 and 14 d.

**Table 4:** Effect of sex category and slaughter age on meat quality characteristics of the *longissimus thoracis and semitendinosus* muscles of Brangus cattle

Muscle	M16		M20		SE	Significance			
	CM	NCM	CM	NCM	M	S	SA	S X SA	
LT	Sarcomere length, $\mu\text{m}$	2.00	2.07	1.96	2.01	0.02	ns	ns	ns
	Intramuscular fat (g of lipids <sup>-1</sup> fresh tissue)	2.82	2.22	2.49	1.94	0.17	ns	ns	ns
	Total collagen (mg <sup>-1</sup> fresh tissue)	2.13 <sup>b</sup>	2.82 <sup>a</sup>	2.36 <sup>ab</sup>	2.92 <sup>a</sup>	0.12	**	ns	ns
	Soluble collagen (total collagen ratio found as soluble collagen, %)	20.68 <sup>a</sup>	14.18 <sup>b</sup>	14.57 <sup>b</sup>	7.40 <sup>c</sup>	1.19	***	***	ns
	Glycogen (g <sup>-1</sup> fresh tissue, $\mu\text{mol}$ glucose)	103.35 <sup>ab</sup>	89.26 <sup>b</sup>	111.04 <sup>ab</sup>	115.82 <sup>a</sup>	4.34	ns	*	ns
	Myofibrillar fragmentation index	82.08	78.83	87.74	82.66	2.48	ns	ns	ns
ST	Sarcomere length, $\mu\text{m}$	2.26 <sup>a</sup>	2.13 <sup>b</sup>	2.19 <sup>ab</sup>	2.07 <sup>b</sup>	0.05	***	ns	ns
	Intramuscular fat (g of lipids <sup>-1</sup> fresh tissue)	3.80 <sup>ab</sup>	4.04 <sup>a</sup>	3.03 <sup>ab</sup>	2.43 <sup>b</sup>	0.50	ns	*	ns
	Total collagen (mg <sup>-1</sup> fresh tissue)	4.09 <sup>b</sup>	4.90 <sup>a</sup>	4.75 <sup>a</sup>	5.01 <sup>a</sup>	0.22	*	ns	ns
	Soluble collagen (total collagen ratio found as soluble collagen, %)	6.59	5.24	5.35	5.45	0.33	ns	ns	ns
	Glycogen (g <sup>-1</sup> fresh tissue, $\mu\text{mol}$ glucose)	97.97	112.14	92.04	94.98	3.14	ns	ns	ns
	Myofibrillar fragmentation index	81.21	71.34	89.03	84.53	2.57	ns	ns	ns

M16= males slaughtered at 16 mo; M20= males slaughtered at 20 mo; CM= castrated males; NCM= non-castrated males; SEM= standard error of the mean; S= sex category; SA= slaughter age; S x SA= interaction between sex category and slaughter age; LT: *Longissimus thoracis*; ST: *semitendinosus*.

<sup>abc</sup> LS-means with different superscripts within a row are statistically different ( $P < 0.05$ ). \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ; ns:  $P > 0.1$

## Discussion

The trial revealed an expected outcome as non-castrated animals exhibited greater increases in both live weight and hot carcass weight than castrated at older ages<sup>(23)</sup>. This can be attributed to the higher levels of testosterone observed in non-castrated animals, which were also reflected in their larger ribeye areas. The absence of variations in dressing percentage, adjusted by live weight, between treatments can be attributed to the lack of disparities in backfat thickness across different ages. Additionally, the differences observed between castrated and non-castrated animals in BFT were not significant enough to account for any significant variation in dressing percentage. These findings are consistent with the conclusions drawn by other researchers who have conducted similar studies<sup>(23,24)</sup>.

The study revealed that the variations in ribeye area and backfat thickness between different sex categories had an impact on the decline of LT muscle temperature<sup>(25)</sup>. However, despite lower temperatures observed in non-castrated animals, no differences in sarcomere length were found between sex categories in the LT muscle. Furthermore, although there were differences in sarcomere length in the ST muscle between sex categories, the temp@pH 6 remained above 12 °C for both sex categories, which was suggested as the minimum threshold to avoid shortening and meat toughening<sup>(4,26)</sup>, in agreement with previous records<sup>(2)</sup>.

The castration of Brangus males led to a reduction of the WBSF for the LT steaks, as reported by other authors<sup>(2,5,27)</sup>. This result was in line with the lower TC content as well as the higher SC content observed in the LT muscle of CM than in that of NCM. This different content of TC and SC could be attributed to a lower testosterone level in castrated than in non-castrated cattle<sup>(8)</sup>.

Aging the muscles for 14 d instead of 2 d resulted in a higher improvement in WBSF for the LT muscle<sup>(5)</sup>. It is known that the LT muscle is highly influenced by myofibril degradation<sup>(28)</sup>. The association between MFI and TC with WBSF suggests that, at 2 d, the differences in WBSF in LT muscle were associated with differences in proteolytic activity; however, at 14 d, the existing correlation with TC would indicate that the differences in proteolytic activity would no longer have an effect i.e., proteolysis could have been completed, so differences in WBSF would be due to differences in connective content<sup>(5,29)</sup>.

In present study, castration and slaughter age treatments did not affect WBSF values for ST steaks<sup>(5)</sup>. This could be due to the high TC content of this muscle as compared to other muscles and the positive correlation found between TC content and WBSF of ST muscle. It has been proposed<sup>(5,9)</sup> that collagen content would be the major factor affecting meat tenderness and that it might mask any potential improvement due to other effects.

In the present study, in agreement with findings reported by other authors<sup>(1,7)</sup>, the higher L\* in both muscles observed in younger castrated animals were related to the lower pH and temperature decline of the former<sup>(28)</sup>, and probably to the increasing myoglobin content with age and testosterone<sup>(29)</sup>. On the other hand, the absence of variation in color variables of aged LT muscle in older animals might be attributed to the increased color parameter values due to *postmortem* aging, which could reduce differences among animal treatments<sup>(30)</sup>. In the case of unaged samples of ST muscle, the higher levels of yellowness and redness observed in older animals<sup>(29)</sup> can be attributed to the accumulation of myoglobin pigments as age progresses<sup>(31,32)</sup>. Additionally, this phenomenon may also be influenced by the higher pH values observed in M20<sup>(31)</sup>. Nevertheless, at 14 d, as a consequence of the *postmortem* aging and the decreased color stability<sup>(33)</sup>, these differences were not observed, except for the b\* in M16, which were only 5 % higher than in M20. The latter could be associated with a higher metmyoglobin content in the M16 aged meat<sup>(30)</sup>.

Since bulls are more susceptible to pre-slaughter stress than steers, their probabilities to produce meat with higher pHu and dark meat are also higher<sup>(34)</sup>. In the current study, the pHu of bulls was slightly higher than that of steers, but no dark meat was observed; the pHu was within the optimal range<sup>(31)</sup> (5.4-5.7).

## Conclusions and implications

Irrespective of the age at slaughter, the slaughtering of non-castrated males resulted in an increase in hot carcass weight and ribeye areas. However, backfat thickness decreased compared to castrated males. Regardless of the manipulation of castration or age at slaughter, the dressing percentage remained unaffected. The effects of castration and slaughter age of Brangus males on the meat quality characteristics differ in the different muscles evaluated. Muscles with high amount of connective tissue as ST did not generate differences in WBSF irrespective of the treatments. In contrast, muscles with low amount of connective tissue as LT was affected by castration and age of slaughter associated to pHu, myofibrillar fragmentation index, total collagen and soluble collagen content. Castration produced lighter colors in both muscles associated to pHu and myoglobin content.

## Acknowledgments and conflict of interest

This work is part of the Doctoral Dissertation of the senior author at the Graduate Program in Agricultural Sciences, Faculty of Agricultural Sciences, National University of Mar del Plata, Argentina. This research was funded by Nacional Institute of Agricultural Technology (INTA), Argentina and Research Council from Catholic University of Salta, Argentina (UCASAL) (RR N° 694/2012, 1294/2015). Support for this research project was also

provided by Bermejo SA and San Pablo Alberdi SA, Salta Province, Argentina. We certify that there is no conflict of interest.

**Literature cited:**

- 1-Marti S, Realini CE, Bach A, Perez–Juan M, Devant M. Effect of castration and slaughter age on performance, carcass, and meat quality traits of Holstein calves fed a high-concentrate diet. *J Anim Sci* 2013;(91):1129–114.
- 2-Silva LHP, Rodrigues RTS, Assis DEF, Benedetti PDB, Duarte MS, Chizzotti ML. Explaining meat quality of bulls and steers by differential proteome and phosphoproteome analysis of skeletal muscle. *J Proteom* 2019;(199):51–66.
- 3- Steen RWJ. The effect of plane nutrition and slaughter weight on growth and food efficiency in bulls, steers and heifers of three breed crosses. *Livest Prod Sci* 1995;(42): 1-11
- 4-Page JK, Wulf DM, Schwotzer TR. A survey of beef muscle color and Ph. *J Anim Sci* 2001;79(3):678-87.
- 5-Rodriguez J, Unruh J, Villarreal M, Murillo O, Rojas S, Camacho J. Carcass and meat quality characteristics of Brahman cross Bulls and steers finished on tropical pastures in Costa Rica. *Meat Sci* 2014;(96):1340–1344.
- 6-Weston AR, Rogers PRW, Althen TG. Review: the role of collagen in meat tenderness. *Prof Anim Sci* 2002;18(2):107-111.
- 7-Nian Y, Kerry JP, Prendiville R, Allen P. The eating quality of beef from young dairy bulls derived from two breed types at three ages from two different production systems. *Irish J Agric Food Res* 2018;56(1):31-44.
- 8-Sadowska A, Swiderski F, Rakowska R, Nogalski Z. The quality of steer and bull meat obtained by crossing Holstein – Friesian cows with Charolais bulls. *Pak J Agr Sci* 2017;(54):899-905.
- 9-Rhee MS, Wheeler TL, Shackelford SD, Koohmaraie M. Variation in palatability and biochemical traits within and among eleven beef muscles. *J Anim Sci* 2004;82(2):534–550.
- 10-Starkey CP, Geesink GH, Oddy VH, Hopkins DL. Explaining the variation in lamb *longissimus* shear force across and within ageing periods using protein degradation, sarcomere length and collagen characteristics. *Meat Sci* 2015;(105):32–37.

- 11-Mazzucco JP, Melucci LM, Villarreal EL, Mezzadra CA, Corva P, Motter MM, *et al.* Effect of ageing and  $\mu$ -calpain markers on meat quality from Brangus steers finished on pasture. *Meat Sci* 2010;(86):878–882.
- 12-dos Santos MD, de Almeida Rego FC, da Silva JM, Costa DS, de Souza CN, Santana JL. Rendimento e acabamento da carcaça de novilhos inteiros e castrados da raça Brangus terminados em confinamento. *Rev Bras Hig San Anim* 2014;8(3):62–71.
- 13-Elzo MA, Johnson DD, Wasdin JD. Driver Carcass and meat palatability breed differences and heterosis effects in an Angus–Brahman multibreed population. *Meat Sci* 2012;(90):87–92.
- 14-Seenger J, Nuernberg G, Hartung M, Szucs E, Ender K, Nuernberg K. ANKOM – a new instrument for the determination of fat in muscle and meat cuts – a comparison. *Arch Tierz Dummerstorf* 2008;51(5):449-457.
- 15-Warner–Bratzler shear-force American Meat Science Association (AMSA). 1995.
- 16-Bergman I, Loxley R. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. *Ann Chem* 1963;(35):1961–1965.
- 17-Hill HF. The solubility of intramuscular collagen content in meat animals of various ages. *Food Sci* 1966;(31):161–166.
- 18-Cross HR, West RL, Dutson TR. Comparison of methods for measuring sarcomere length in beef muscle. *Meat Sci* 1981;(5):261-266.
- 19-Monin G, Sellier P. Pork of low technological quality with a normal rate of muscle pH fall in the immediate *postmortem* period: The case of the Hampshire breed. *Meat Sci* 1985;(13):49-63.
- 20-Pighin DG, Davies P, Grigioni G, Pazos AA, Ceconi I, Mendez D, Buffarini M, Sancho A, Gonzalez CB. Effect of slaughter handling conditions and animal temperament on bovine meat quality markers. *Arch Zootec* 2013;(62):399–404.
- 21-Neath KE, Del Barrio AN, Lapitan RM, Herrera JRV, Cruz LC, Fujihara T, Muroya S, Chikuni K, Hirabayashi M, Kanai Y. Difference in tenderness and pH decline between water buffalo meat and beef during *post-mortem* ageing. *Meat Sci* 2007;(75):499–505.

- 22-Hopkins DL, Martin L, Gilmour AR. The impact of homogenizer type and speed on the determination of myofibrillar fragmentation. *Meat Sci* 2004;(67):705-710.
- 23- Kuss F, López J, Jardim-Barcellos JO, Restle J, Moletta LJ, Perotto D. Características da carcaça de novilhos não-castrados ou castrados terminados em confinamento e abatidos aos 16 ou 26 meses de idade. *R Bras Zootec* 2009;38(3):515-522.
- 24- Restle J, Vaz FN. Eficiência e qualidade na produção de carne bovina. In: Reuniao Annual da Sociedade Bras de Zootec. Santa Maria. 2003:40.
- 25-Aalhus JL, Janz JAM, Tong AKW, Jones SDM, Robertson WM. The influence of chilling rate and fat cover on beef quality. *Can J Anim Sci* 2001;8(3):321-330.
- 26-Battaglia C, Vilella GF, Bernardo APS, Gomes CL, Biase AG, Albertini TZ, Pflanzler SB. Comparison of methods for measuring shear force and sarcomere length and their relationship with sensorial tenderness of *longissimus* muscle in beef. *J Texture Stud* 2019;(51):252–262.
- 27-Fitzpatrick LA. Growth and meat quality of grain finished entire male *Bos indicus* cattle. Project code: B.NBP.0486. M & Liv Austr Lim, North Sydney, Australia; 2014.
- 28-Koohmaraie M, Matthew PK, Shackelford SD, Veiseth E, Wheeler TL. Meat tenderness and muscle growth: is there any relationship? *Meat Sci* 2002;(62):345–352.
- 29-Wright SA, Ramos P, Johnson DD, Scheffler JM, Elzo MA, Mateescu RG, Bass AL, Carr CC, Scheffler TL. Brahman genetics influence muscle fiber properties, protein degradation, and tenderness in an Angus-Brahman multibreed herd. *Meat Sci* 2017;(135):84-93.
- 30-Gil M, Serra X, Gispert M, Angels OM, Sañudo C, Panea B, *et al.* The effect of breed-production systems on the myosin heavy chain 1, the biochemical characteristics and the color variables of *longissimus thoracis* from seven Spanish beef cattle breeds. *Meat Sci* 2001;(58):181–188.
- 31-Ijaz M, Jaspal MH, Hayat Z, Yar MK, Badar IH, Ullah S, *et al.* Effect of animal age, *postmortem* chilling rate, and aging time on meat quality attributes of water buffalo and humped cattle bulls. *Anim Sci J* 2020;(91):e13354.
- 32-Hughes JM, Clarke FM, Purslow PP, Warner RD. Meat color is determined not only by chromatic heme pigments but also by the physical structure and achromatic light scattering properties of the muscle. *Compr Rev Food Sci Food Saf* 2019;(19):44–63.

- 33-Oliete B, Moreno T, Carballo JA, Varela A, Monserrat L, Sánchez L. Influence of ageing time on the quality of yearling calf meat under vacuum. *Eur Food Res Technol* 2005;(20):489–493.
- 34-Duarte MS, Paulino PVR, Fonseca MA, Diniz LL, Cavali J, Serão NVL, *et al.* Influence of dental carcass maturity on carcass traits and meat quality of Nellore bulls. *Meat Sci* 2011;(88):441–446.