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Research Article

Beef of Nellore cattle has limited tenderization despite pH decline in *Longissimus lumborum*

Patricia Maloso Ramos[1]*, Priscila Robertina dos Santos-Donado[2], Giuliana Micai de Oliveira[1], Carmen Josefina Contreras–Castillo[2], Tracy L. Scheffler[3], Saulo da Luz e Silva[4], Luciane Silva Martello[5], Eduardo Francisquine Delgado[1]

[1]Universidade de São Paulo/ESALQ – Depto. de Zootecnia, C.P. 09 – Piracicaba, SP –Brasil.
[2]Universidade de São Paulo/ESALQ – Depto. de Agroindústria, Alimentos e Nutrição – Piracicaba, SP – Brasil.
[3]University of Florida /IFAS – Dept. of Animal Sciences, 2250 Shealy Dr. – 32611 – Gainesville, FL – USA.
[4]Universidade de São Paulo/FZEA – Depto. de Zootecnia, Av. Duque de Caxias Norte, 225 – 13635–900 – Pirassununga, SP – Brasil.
[5]Universidade de São Paulo/FZEA – Depto. de Engenharia de Biossistemas – Pirassununga, SP – Brasil.

*Corresponding author <ramospm@usp.br>

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ABSTRACT: *Bos taurus indicus* temperament is variable and affects beef tenderization. Our objective was to investigate temperament and performance of non–castrated Nellore and identify groups based on *Longissimus lumborum* (LL) pH decline as well as beef characteristics produced by those groups. We investigated 94 animals with a subset of carcasses (n = 24) selected based on LL pH at 24 h *postmortem* (*pm*) to represent two groups: resistant to pH decline (> 5.8 called pH–Res; n = 10) and normal (< 5.7 called pH–Nor; n = 14). Steaks were fabricated from the LL muscle and randomly assigned to aging (2, 7, 14, and 21 days). Sarcomere length, cooking loss, myofibrillar fragmentation index (MFI), and Warner–Bratzler shear force (WBSF) were determined. Data on temperament were investigated in a multivariate approach, while beef data were compared between groups using the analysis of variance. Rectal temperature at the beginning of the finishing phase and total weight gain were greater and related to animals in the pH–Res group ($p < 0.01$ and $p = 0.03$, respectively). Temperature and pH decline curves, sarcomere length, and cooking loss revealed that pH–Res produced beef with lower quality compared to the pH–Nor group. Results for MFI and WBSF did not show differences between groups within each time *pm*; however, overall steaks from pH–Res were tougher ($p = 0.06$). Incidence of LL pH between 5.8 and 5.9 at 24 h *pm* did not compromise the tenderization rate or extension; however, it affected the water holding capacity in this population of Nellore cattle.

Keywords: pH, cooking loss, quality, sarcomere, temperament

Introduction

Genetically, *Bos taurus indicus* subspecies has greater ability to cope with tropical conditions compared to non–adapted *Bos taurus taurus*, but at the expense of reduced beef quality (Wheeler et al., 1996). In Brazil, producers choose Nellore breed due to the adaptability of animals, mostly raised under low–controlled pasture conditions. Traditionally, meat toughness in Nellore cattle is associated to greater calpastatin activity (Whipple et al., 1990); nevertheless, animal behavior can also influence meat tenderness. Nellore cattle has different temperament under similar conditions. More excitable steers are associated to greater sympathetic tonus and consequent greater stimulation of hypothalamic–pituitary–adrenal axis resulting in beef with reduced tenderness (Coutinho et al., 2017). Meat toughness of excitable Nellore steers is not resolved, even after aging (Souza et al., 2019). Additionally, excitable animals can have reduced concentration of muscle glycogen at slaughter, which limits adenosine triphosphate (ATP) levels *postmortem* (*pm*) and consequently hindering the pH decline. The pH rates and temperature decline have a significant effect on proteases activation and consequently beef quality (Hwang and Thompson, 2001).

Subcutaneous fat deposition is a challenge in non–castrated Nellore and is lower than in castrated animals, regardless of the castration age (Anaruma et al., 2020). The lower fat thickness may lead to rapid rate of colling and can favor excessive sarcomere cold shortening. Carcasses with reduced fat thickness and pH higher than six (6.0) at a temperature lower than 10 °C have all the prerequisites for an excessive shortening of sarcomeres (Thompson, 2002), with potential to reduce meat tenderization when compared to carcass that did not undergo these conditions (Fausto et al., 2017).

Desirable beef tenderness (values lower than 44 N) achieved in earlier *pm* (i.e. after 14 days of aging) is a challenge for the Brazilian meat industry. This study investigated non–castrated Nellore temperament and performance during the finishing phase at feedlot, as well as beef quality of a sub–set selected based on the pH at 24 h *pm*. The hypothesis is that beef from those groups have a similar tenderization rate and extension during 21 days of aging, since the pH is not the limiting factor for proteolysis in indicine cattle.

Materials and Methods

The procedures were approved by the Ethics Committee on the Use of Animals (protocol number 14.1.1370.74) from the College of Animal Science and Food Engineering, University of São Paulo (FZEA/USP), Pirassununga, São Paulo State, Brazil.

Animals, facilities, and diets

Non–castrated Nellore (*Bos taurus indicus*; n = 94) with an average age of 20 ± 2 months, born and raised on pasture were selected to be transferred to feedlots

(Pirassununga, São Paulo State, Brazil, 21°59′46″ S, 47°25′33″ W, altitude 627 m). At the beginning of the experimental phase, animals had an average of 402 ± 32.2 kg of live weight and were randomly assigned to one of the feedlot systems (collective or individual pens). One system used collective pens (10 × 23 m^2) with 12 animals per pen (n = 47; one pen had 11 animals), which were equipped with Calan Broadbent feeding gates. The second system used individual pens (5 × 8 m^2; n = 47). Automatic drinkers and covered feeders were provided to all animals regardless of the pen type during all the experimental period. Animals were part of a nutrition experiment, which evaluated the effect of three levels of exogenous fibrolytic enzyme (0, 0.37, and 0.74) inclusion to the concentrate, associated to two particle sizes of corn (thin or thick). The basal diet contained corn silage as roughage source (21.8 % of dry matter) and corn grain, citrus pulp, urea, mineral salt, limestone plus enzymes as concentrate source (78.2 % dry matter). All diets were provided *ad libitum* after an adaptation period of 15 days and the feedlot period average was 75 days. At slaughter, animals had an average live weight of 489 ± 38.3 kg.

Handlings, animal temperament, physiology, and performance variables

Animals were evaluated at the beginning of the experimental phase, after the adaptation period (handling 1), and at the end (handling 2 – 2 days before slaughter). For each animal, weight gain (WG) was calculated as the difference between final and initial body weights. Trained professionals assessed animal temperament, as previously described (Coutinho et al., 2017). In brief, for both handlings, the chute score (CS; scale index), exit velocity (EV; m s^{-1}), and rectal temperature (RT; °C) were determined. Chute scores were assigned according to 1 to 4 scale, where 1 = no body or head movement, ears, and tail relaxed; 2 = some movement with head raised and ears up; 3 = frequent movement but not vigorous, head, ear, and tail movements, and possible vocalization; 4 = offers great resistance, abrupt and vigorous movements of the entire animal as well as the head, ear, and tail, audible breathing, with possible jumping or falling. The score was attributed immediately after the animal entered the chute by the same evaluator on both occasions. The rectal temperature was obtained immediately after the score. For each handling, the average temperament index (AT) was calculated using the chute score and exit velocity [AT = (EV + CS)/2] (King et al., 2006). During the second handling, subcutaneous fat thickness (SFT; mm) between the 12th and 13th ribs was determined using ultrasound with linear transducer of 3.5 MHz and 172 mm long.

Slaughter, temperature, and pH decline

As part of the experimental procedures, animals were fed before slaughter to collect part of ruminal contents for the nutritional experiment. Therefore, animals were not in a fasted state. On the day of slaughter, animals were transported by truck early in the morning from the feedlot to the *campus* slaughter facility, around 400 m from the feedlot, where they were unloaded and waited with free access to water. Animals were slaughtered under the regulations prescribed by São Paulo State. Due to technical limitation of the facility, four groups (2 groups with 24, and 2 with 23 animals; n = 94) were formed and assigned to one of the four slaughter days. All slaughters were conducted following the same procedures, which included stunning with a captive bolt and exsanguination by severing of the carotid artery and jugular vein.

Before carcasses were ready to be placed in the cold room (approximately 1 h after exsanguination), the LL muscle in the left side of each carcass was assessed by the insertion of a pH meter probe between the 12th and 13th ribs, accompanied by a thermometer probe. The pH meter was calibrated according to manufacturer recommendation and considering carcass temperature (i.e., when carcasses were still hot, calibration buffers were kept at room temperature and when carcasses were cold, buffers were also cold for calibration). Calibration was performed before each time *pm* and buffer 7 was used as a quality control reading after assessing five carcasses. When buffer 7 reading was off by ± 0.05, a new calibration was performed before measuring the LL pH. Before every measurement, the probe was placed in a new position within LL muscle and kept close to the first insertion (between the 12th and 13th ribs). This approach is necessary to avoid damaged tissue that releases fluid, which could lead to an incorrect estimation of muscle pH. Using the same probe, measurements were conducted multiple times *pm* to represent pH and temperature decline (3, 6, 9, and 24 h) during all slaughter days.

At 24 h *pm*, LL that showed resistance to pH decline, evidenced by values above 5.8, were selected for sampling and forming the pH–Res group. The comparison group comprised the LL with pH values lower than 5.7 at 24 h *pm* (pH–Nor group). The third slaughter did not have any occurrence of LL with pH above the pre-established threshold; therefore, no carcass was sampled that day. Table 1 shows the number of carcasses sampled and average pH (± SD) by slaughter day. While animals in first and second slaughters were exclusively from individual pens at feedlot, animals in the fourth slaughter were all from collective pens. Therefore, groups had similar percentage of animals representing each feedlot system.

At 24 h *pm*, the LL muscle from the selected carcasses (n = 24) was excised a 2.5 cm steak was cut from the cranial portion and sampled.

Analysis 24 h *postmortem*

Sarcomere length

Sarcomere length was determined in five distinct anatomic positions to represent the entire steak (Fausto

Table 1 – *Longissimus lumborum* pH at 24 h *postmortem* by slaughter day and within the group of interest measured in carcasses from non–castrated Nellore finished at feedlot[1].

	pH < 5.7		pH > 5.8	
	Number	Mean ± SD	Number	Mean ± SD
Slaughter 1	4	5.41 ± 0.01	2	5.89 ± 0.09
Slaughter 2	6	5.56 ± 0.06	6	5.89 ± 0.08
Slaughter 4	4	5.47 ± 0.04	2	5.83 ± 0.03
Sub–total	14	5.49 ± 0.08	10	5.88 ± 0.07
Total	24	5.65 ± 0.21		

[1]pH decline (1, 3, 6, 9 and 24 h) was accompanied in a larger set of animals (n = 94). Animals were not in the fasted state and were slaughtered in an experimental abattoir (under São Paulo State regulation) belonging to the University of São Paulo (Pirassununga, SP, Brazil). Slaughter 3 is not shown on table because it did not have any occurrence of muscle pH above 5.8.

et al., 2017). In brief, the steak anatomical positions were lateral, medial, dorsal, ventral, and central. A muscle piece from each position was wrapped in aluminum foil, snap frozen, and kept in nitrogen until transferred to an ultra–freezer (– 80 °C). Muscles were immersed, thawed in 5 % glutaraldehyde solution (100 mM $NaHPO_4$ buffer, pH 7.2), and homogenized. Samples were incubated inside the refrigerator for at least 4 h and then washed and fixed with 0.2 M sucrose solution (Cross et al., 1981). Drops of homogenate were deposited onto one slide per steak position, covered with coverslips, and assessed at ten positions under a Nikon Eclipse 80i light microscope using 100 × amplification lens and Nikon Elements F software. The average of the ten measurements per slide represented one anatomic position and the average of the five positions represented the sarcomere length (µm) for each steak.

Aging analysis (2 to 21 days *postmortem*)

The remaining LL muscle, approximately 15 cm long, was placed unpackaged on a rack and transferred to a cold room (3 ± 2 °C). At 48 h *pm*, starting from the cranial portion of the LL muscle, four additional 2.5 cm steaks were fabricated and randomly assigned to four aging periods (2, 7, 14, and 21 days), vacuum packed, and transferred to a box filled with ice to be transported from the slaughter facility to the laboratory (Piracicaba, São Paulo State, Brazil). The transport lasted 01h30 and the steaks remained in the box with ice during this time. Steaks were aged in a temperature–monitored cold room (3 ± 2 °C), but never frozen.

Myofibrillar fragmentation index (MFI)

At each time *pm*, approximately 5 g of muscle was excised from each steak, cut in smaller pieces, immediately frozen using liquid nitrogen, and transferred to ultra–freezer (–80 °C) until analysis. All samples were assessed in duplicates. From the 5 g, 1 g of muscle was cut and transferred to thaw in a falcon tube containing 10 mL of cold phosphate buffer (10 mM KH_2PO_4, 10 mM K_2HPO_4, 1 mM EDTA, 1 mM $MgCl_2 \cdot 6H_2O$, 100 mM KCl, pH 7 at 4 °C). Myofibrils were extracted and washed by repeating three cycles of homogenization at 16,000 rpm per 30 s followed by 30 s of resting on ice, with a centrifugation at 1,000 × g for 10 min. After the centrifugation, the pellet was resuspended by adding 5 mL of same extraction buffer and the solution was filtered to remove connective tissue debris. The sub–sample set was prepared by dilution with 1 M NaOH, protein concentration was determined using biuret (6 mM $CuSO_4 \cdot 5H_2O$, 20 mM $NaKCu_4H_4O_6 \cdot 4H_2O$, 75 mM NaOH) as color reagent, and bovine serum albumin (BSA) as protein standard curve (0 to 6 mg mL^{-1}). Final samples were diluted to 0.5 mg of protein mL^{-1} using the extraction buffer and absorbance was determined at 540 nm spectrophotometrically. The final index was calculated by multiplying the absorbance reading by 200 (Culler et al., 1978).

Cooking loss

Steaks were weighed before cooking to determine shear force. Cooked steaks were cooled at room temperature, plastic wrapped, and then placed in a refrigerator (3 ± 2 °C) overnight. The final weight was determined on chilled steaks immediately before cores were taken for WBSF. Cooking loss was calculated by the difference between initial and final weights and converted to percentage. Some information was lost for the 2-day-steaks (n = 18).

Warner–Bratzler shear force (WBSF)

Steaks were cooked in open grills and the temperature was monitored by the insertion of the thermometer at the center until it reached 71 °C. Steaks were cooled as previously indicated and six uniform round cores (1.27 cm diameter) were taken from each steak. Cores were parallel to longitudinal direction of muscle fibers and areas with connective tissue were avoided (AMSA, 2015). Cores were sheared using Warner–Bratzler shear 'V slot blade' coupled with a digital system at a crosshead speed of 200 mm min^{-1}. The simple average from the six cores represents steak WBSF that was expressed in Newtons (N).

Statistical analysis

All data were analyzed using SAS (Statistical Analysis System software, version 9.4, 2018). Nine variables (CS 1 and 2, EV 1 and 2, AT 1 and 2, RT 1 and 2, and WG) from 94 animals were used to investigate data variation in a multivariate approach. The model used to generate the residual matrix for the principal component analysis considered a complete randomized block design with block as feedlot system, fixed effects of level of fibrolytic enzyme, as well as corn granulometry and their interaction, and animal within the block considered as random effect. After generating the eigenvectors, the temperament index (TI) was created.

The temperament index considered the four highest eigenvectors attributed to the variables to form the first principal component. Therefore, the formula considered the exit velocity and the average temperament index from both handlings, represented as:

$$TI = (AT1 \times 0.50) + (EV2 \times 0.46) + (EV1 \times 0.45) + (AT2 \times 0.29)$$

The index was calculated for each of the subsequent selected animal (n = 24). Using this data subset, differences between the groups (pH–Nor *vs.* pH–Res) were investigated in a mixed model and a complete randomized block design with slaughter date as block, and effects of corn particle size and level of fibrolytic enzyme inclusion on concentrate considered on the model, while the animal was the random effect. The least square means were compared by the Tukey–Kramer test at 5 % significance.

Meat quality data (n = 24) from steaks in the selected groups (pH–Res *vs.* pH–Nor) were compared using a mixed model, a completely randomized block design, with slaughter date as block, effects of corn particle size and level of fibrolytic enzyme inclusion on concentrate considered on the model, while the animal order at slaughter was considered as a random effect. The fixed effects of pH group (pH), aging period (t; time *postmortem*) and interaction (pH × t) were tested, with time considered as a repeated measure. For the pH analysis, the temperature was included as a random effect to the model. The least square means were compared by the Tukey–Kramer test at 5 % significance.

Results and Discussion

Animal temperament, rectal temperature, and performance

The first three principal components using data from 94 non–castrated Nellore animals and nine variables, with measurements in two handlings during feedlot period, explained approximately 70 % of residual data variation (Table 2). The plot showing component pattern for principal components 1 and 2 (Figure 1), confirmed

Table 2 – Principal component (PC) analysis of temperament indicators of non–castrated Nellore (n = 94)[1].

Component	Eigenvalues	Cumulative variance (%)	Eigenvector			
			AT1	EV2	EV1	AT2
PC1	2.99	33.26	0.50	0.46	0.45	0.29
PC2	1.98	55.25	–0.17	0.23	–0.04	0.60
PC3	1.28	69.49	–0.32	–0.06	–0.41	0.04

[1]Temperament was assessed in two handlings by trained professional (beginning and end of finishing phase at feedlot); Nine variables were used: chute score (CS) 1 and 2; exit velocity (EV) 1 and 2; rectal temperature (RT) 1 and 2; average temperament index (AT) 1 and 2 [AT = (EV + CS)/2]; and total weight gain.

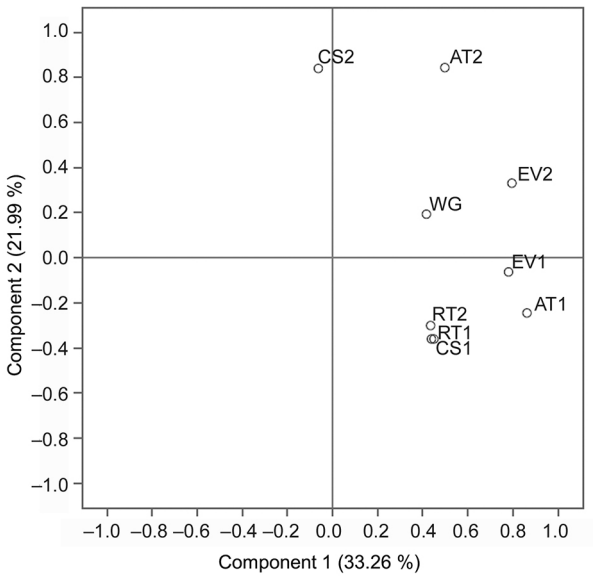

Figure 1 – Components pattern from principal component analysis using information from 94 non–castrated Nellore. Component 1 (x axis) and 2 (y axis) explained 55.25 % of residual data distribution. EV = exit velocity; CS = chute score; AT = average temperament index (EV + CS/2); RT = rectal temperature; WG = weight gain; 1 and 2 refers to handlings during the feedlot period (1 = initial and 2 = 2 days before slaughter).

that AT1, EV1, and EV2 represented most data residual distribution, noting that AT1 is an average between EV1 and CS1. Coutinho et al. (2017) demonstrated that temperament indicators from handling prior to slaughter (handling 2) had higher correlation coefficients with WBSF and tenderization extension than indicators from the first handling. The current study partially agreed with that, since EV2 showed the second highest eigenvector value (0.46). However, the highest eigenvector in our study was attributed to AT1 (0.50). Therefore, when evaluating animal temperament, measurements should be taken at multiple times during the finishing phase. The greater importance attributed to AT1 in our study could be explained by the relevance of the novelty fear when handling animal raised extensively and later transferred to feedlot. This average index considered CS and EV measured in the first handling, after animals were transferred from pasture to feedlot and adapted to diets. Since fear is a strong stressor and can be enhanced by exposure to a new environment, mainly in animals that have little contact with people and handling procedures. After a training and handling period, the animals can be acquainted (Grandin et al., 1998). Possibly, animals were acquainted with handlers and handling procedures from the first to second handling, which could explain the reduction of eigenvector from 0.50 to 0.29 (AT1 to AT2, respectively; Table 2).

Animal information within the pH groups

Comparison between the pH groups showed that most temperament data were similar (Table 3). However, animals in the pH–Res group showed higher RT1 ($p < 0.01$). The difference was not kept during the experiment. Increased rectal temperature can be the result of activation by stressors of the integrated neuroendocrine axes hypothalamic–pituitary–adrenal and the sympathoadrenal, with long term and short–term responses, respectively. Acute stress during chute restraint is correlated to increased heart rate, rectal temperature, serum cortisol, plasma lactate, and serum creatine kinase at slaughter (Gruber et al., 2014). Additionally, excitable cattle possibly keeps greater basal blood cortisol concentrations than calm animals do (Curley et al., 2008). Therefore, excitable cattle can express the combined effects and sustain greater basal cortisol levels because of the hypothalamic–pituitary–adrenal stimulus, as well as greater response to acute stress by sympathoadrenal axis.

Animals in the pH–Res group showed a higher DMI ($p = 0.09$) reflected in greater WG ($p = 0.03$; Table 3) when compared to pH–Nor group. Animals in the pH–Res group gained approximately 20 kg more during the finishing phase than animals in the pH–Nor group. However, both groups had similar feed efficiency ($p = 0.38$) and weight at slaughter ($p = 0.30$). In part, greater WG could be explained by lower ($p = 0.09$) average weight at the beginning of the finishing phase in animals selected to the pH–Res group. However, greater WG in animals in pH–Res group, which also showed greater RT1, is not in agreement with previous findings that *Bos indicus* crosses with more excitable temperament displaying reduced average daily gain (Voisinet et al., 1997).

Table 3 – Temperament (EV, CS, AT and TI), physiological (RT) and performance (WG) variables from non–castrated Nellore that produced carcasses with distinct *Longissimus lumborum* pH decline[1].

Variable	Unit	pH group		*p* value
		pH–Nor (n = 14)	pH–Res (n = 10)	
EV1	m s^{-1}	2.57 ± 0.34	2.20 ± 0.38	0.48
EV2		0.99 ± 0.39	1.55 ± 0.38	0.66
CS1	score	1.63 ± 0.31	2.11 ± 0.35	0.31
CS2		1.67 ± 0.27	1.51 ± 0.30	0.31
AT1	index	2.10 ± 0.20	2.16 ± 0.22	0.85
AT2		1.50 ± 0.17	1.68 ± 0.18	0.41
TI	index	3.22 ± 0.39	3.37 ± 0.42	0.69
RT1	°C	37.81 ± 0.17	38.73 ± 0.20	< 0.01
RT2		38.30 ± 0.29	38.35 ± 0.29	0.86
WG	kg	40.99 ± 10.14	62.65 ± 9.92	0.03

[1]Least square means ± standard error; EV = exit velocity; CS = chute score; AT = average temperament index (EV + CS/2) and TI = temperament index (generated using principal component analysis from 94 animals; TI = 0.49 × AT2 + 0.48 × EV2 + 0.45 × EV1 + 0.44 × AT1; for detailed information read Materials and methods section); RT = rectal temperature; WG = weight gain; 1 and 2 refers to handlings during feedlot period (1 = initial and 2 = two days before slaughter); Groups: pH–Res = pH value > 5.8; pH–Norm = pH value < 5.7 measured in the *Longissimus lumborum* at 24 h *postmortem*.

Longissimus lumborum temperature and pH decline

Temperature decline in the LL muscle was different between the pH groups only at 3h *pm* (pH × t: $p < 0.01$; Table 4). The pH–Nor group showed greater ($p < 0.05$) temperature at 3 h *pm* compared to the pH–Res group. There was no interaction between the pH and time *pm* ($p = 0.12$) for the pH decline. Groups showed difference ($p < 0.05$) at all time points. The value observed at 6 h *pm* for the pH–Res group was similar to the initial pH value for the pH–Nor group. The pH within the pH–Res group could not be differentiated statiscally from 1 h up to 6 h *pm*, while, for the pH–Nor group, it was different during this time frame, confirming the resistance to the pH decline for the pH–Res group.

Muscles assessed during 24 h *pm* were collected from animals with similar genetic background, raised under the same husbandry and management conditions, growth, and finishing system and were slaughtered under same conditions. Therefore, the difference observed for temperature and the pH decline could be considered unusual. Explanation of the resistance to the pH decline for a group that represents 10% of the batch of animals is intricate and may involve muscle characteristics, development, and intrinsic energy metabolism, which could be more evident in indicine cattle. During muscle to meat transformation, keeping muscle ATP levels for a longer time can delay the pH decline *pm* (Marsh, 1954). Therefore, the pH–groups potentially differ in their capacity to keep ATP levels early *pm*, as muscle glycogen was not different (data not shown), which was expected since animals were

Table 4 – Temperature and pH decline in *Longissimus lumborum* from non–castrated Nellore finished at feedlot[1].

Variable	Time (h)	pH group		*p* value		
		pH–Nor (n = 14)	pH–Res (n = 10)	pH	Time (t)	pH × t
Temp (°C)	1	38.20 ± 0.34	37.43 ± 0.39	< 0.01	< 0.01	< 0.01
	3	26.57 ± 0.45^{x}	24.42 ± 0.52^{y}			
	6	17.92 ± 0.35	16.51 ± 0.40			
	9	13.49 ± 0.41	12.9 ± 0.46			
	24	6.50 ± 0.26	6.98 ± 0.28			
pH	1	6.65 ± 0.07^{bc}	6.99 ± 0.08^{a}	< 0.01	< 0.01	0.12
	3	6.37 ± 0.08^{cd}	6.79 ± 0.09^{ab}			
	6	6.05 ± 0.07^{de}	6.67 ± 0.09^{abc}			
	9	5.93 ± 0.07^{e}	6.48 ± 0.08^{bc}			
	24	5.49 ± 0.02^{f}	5.86 ± 0.03^{e}			

[1]Least square means ± standard error; Groups: pH–Res = pH value > 5.8; pH–Norm = pH value < 5.7 measured in the *Longissimus lumborum* at 24 h *postmortem*; $^{x-y}$Different letters represent differences ($p < 0.05$) between groups; $^{a-f}$Different letters represent differences ($p < 0.05$) between groups and time *postmortem*.

fed before transportation to the experimental slaughter facility. Additionally, glycolysis could be accelerated by electrical stimulation of carcasses. Electrical stimulation results in faster glycogen depletion, lactate accumulation, and mitochondrial ATP consumption *pm* leading to rapid pH decline, while non-stimulated carcasses have *Longissimus* muscle capable of keeping ATP levels longer, due to mitochondrial contribution (England et al., 2018). Moreover, indicine biological type, represented by the Brahman breed, have higher ATP levels 1 h *pm* when compared to Angus, which is related to slower pH decline, delayed µ-calpain activation and autolysis and consequent reduced proteolysis (Ramos et al., 2020).

The temperature of the *Longissimus* muscle to reach a pH of 6.0 is determinant of cold shortening and it should be between 35 and 12 °C (Hopkins et al., 2014). For the pH-Res group, LL had pH above 6.0 associated to a temperature lower than 10 °C between 9 and 24 h *pm* (Figure 2). Therefore, pH-Res carcasses were exposed to conditions that favors excessive sarcomere shortening (Marsh, 1954; Locker and Hagyard, 1963; Thompson, 2002). Sarcomere length was shorter (p = 0.07) in LL from the pH-Res group compared to pH-Nor (1.76 ± 0.04 and 1.87 ± 0.04 µm, respectively). The subcutaneous fat thickness was similar (p = 0.22) between groups, although the pH-Res groups had numerically less fat than the pH-Nor (2.22 ± 0.40 vs. 2.90 ± 0.35 mm, respectively). Therefore, both groups showed reduced backfat and were at risk of excessive cold shortening, which could compromise proteolysis. In cold-shortened beef, the µ-calpain activity is delayed mostly by the earlier inhibitory calpastatin activity, which promotes a slower tenderization rate (Zamora et al., 1998). According to Smulders et al. (1990), sarcomere length corresponded to 30 % of the variability in panel tenderness score, and values between 1.6 and 1.7 µm received scores between 2 and 6 in an 8-point scale, where 1 is extremely tough. Both groups in our study showed average sarcomere length greater than 1.7 µm. Therefore, sarcomere length in our study potentially did not influence sensory properties or impaired protease activity.

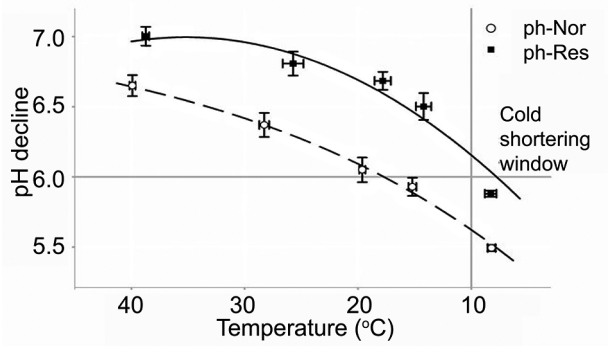

Figure 2 – Temperature (x axis) and pH decline (y axis) curves measured in *Longissimus lumborum* muscle from non-castrated Nellore in two groups selected based on the pH at 24 h *postmortem*: pH-Res = resistant to pH decline (> 5.8) and pH-Nor = normal pH decline (< 5.7).

Beef quality from steaks with distinct pH decline

Cooking loss was influenced by the pH group (pH: p = 0.04; Figure 3). Greater loss was related to steaks from the pH-Nor group aged for 7 days, while lower loss was observed in steaks from the pH-Res group aged for 14 days (33.67 ± 0.97 and 26.13 ± 1.82 %, respectively). Therefore, beef from the pH-Res group aged for 14 days had greater capacity to retain water even after cooking. During the pH decline, muscle structure shrinks and water trapped between the myofibers is forced to the extracellular space driven by the changes in the myofibrillar protein charges (Huff-Lonergan and Lonergan, 2005). This water is ultimately lost as purge. However, if the pH decline is not sufficient to approach the isoelectric point, the water is kept immobilized within the muscle structure. As meat ages or after cooking, this water can be forced out, once the muscle structure changes by proteolysis or heat. Cooking loss can be as high as 45 %, but it also depends on cooking temperature and pH meat. Meat with higher pH has lower cooking loss (Honikel, 2004). Cooking losses observed in our study agree with these observations and overall cooking loss for steaks from the pH-Res was lower (p = 0.04) than steaks from the pH-Nor group. Meat *in natura* with a higher capacity to retain water has a dry appearance because more water is held within the myofilaments and does not move to the surface. Therefore, the visual appearance of this steak is less desirable for consumers.

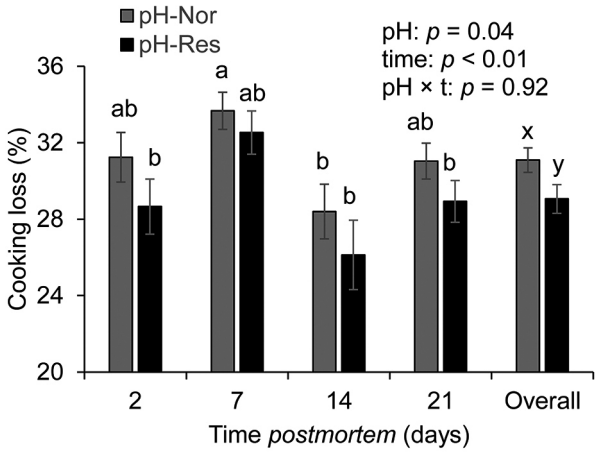

Figure 3 – Cooking loss in steaks of *Longissimus lumborum* muscle from non-castrated Nellore in two groups selected based on pH at 24 h *postmortem*: pH-Res = resistant to pH decline (> 5.8) and pH-Nor = normal pH decline (< 5.7) and aged up to 21 days; $^{a-b}$ Different letters indicate differences (p < 0.05) between groups and time *postmortem*; $^{x-y}$Different letters indicate differences (p = 0.04) between groups.

Proteolysis *pm* results in the breaking down of proteins into small fragments measured by the MFI. Fragmentation and weakening of the muscle structure promotes tenderization, which was instrumentally measured by WBSF. While MFI was not influenced by the pH groups nor interaction (Figure 4), WBSF overall average for pH groups was different (p = 0.06) and higher in pH–Res than in pH–Nor (Figure 5). This is partially in agreement with previous studies showing that intermediate range of ultimate pH could influence the aggregation of small heat shock proteins and myofibrillar proteins, which, in turn, hinders proteolysis by µ–calpain (Lomiwes et al., 2013; 2014). However, the WBSF difference between the pH groups in our study was not observed within the *pm* period. During aging, MFI increased for both groups, as expected, and reached 105.2 ± 6.73 and 100.5 ± 7.57 for pH–Nor and pH–Res, respectively. On the other hand, WBSF showed an unusual pattern, with steaks at 7 d showing lower numerical values than at 14 d. Although the result was unexpected, variability (i.e., standard deviation) in shear force is positively correlated with the shear force average and within–animal variation can be rather large (Dugan and Aalhus, 1998). Since WBSF results were obtained by a small number of animals (n = 24), tenderness variability within–muscle using beef from Nellore cattle needs to be further investigated to understand these findings.

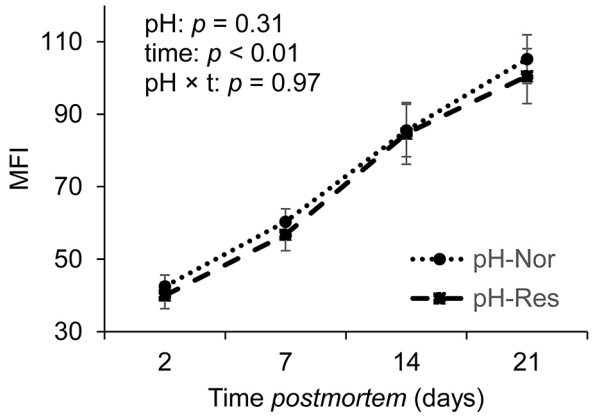

Figure 4 – Myofibrillar fragmentation index (MFI) in steaks of *Longissimus lumborum* muscle from non–castrated Nellore in two groups selected based on the pH at 24 h *postmortem*: pH–Res = resistant to pH decline (> 5.8) and pH–Nor = normal pH decline (< 5.7) and aged up to 21 days.

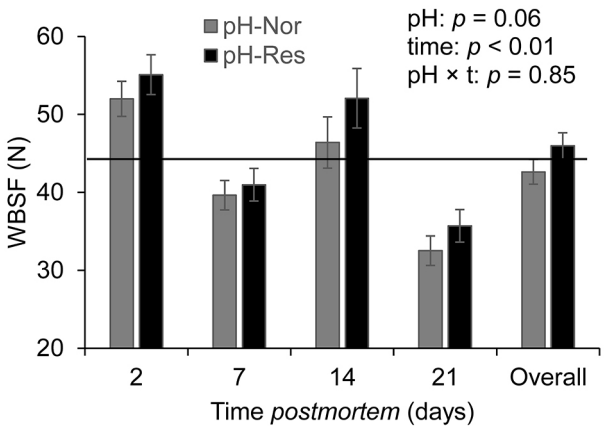

Figure 5 – Warner–Bratzler shear force (WBSF) in steaks of *Longissimus lumborum* muscle from non–castrated Nellore in two groups selected based on the pH at 24 h *postmortem*: pH–Res = resistant to pH decline (> 5.8) and pH–Nor = normal pH decline (< 5.7) and aged up to 21 days.

Considering the tenderness threshold as 44 N (AMSA, 2015), at 7 and 21 days, steaks from both groups could be considered tender. However, 60 % of the steaks from the pH–Res group at 14 days were classified as tough. Conversely, only 30 % of the steaks from the pH–Nor group had the same classification. However, within each *pm* time, steaks from both groups showed similar values, corroborating with the hypothesis that, on average, the tenderization rate and extension considering MFI and WBSF were not influenced by pH.

The lack of differences between groups for MFI (p = 0.31) or within *pm* time for WBSF (p > 0.88) in our study confirmed that the pH at 24 h *pm* and a shorter sarcomere (p = 0.07) were not critical for proteolysis in this population of Nellore cattle. Therefore, effects of resistance of the pH decline were not as apparent in meat from Nellore with inherently reduced proteolysis because of the limitations imposed by the calpastatin inhibitory activity as central contributor and determinant of the tenderization process. Proteolysis *pm* is mostly governed by the µ–calpain activity when calpastatin inhibition is overcome. In indicine cattle, calpastatin is a central player and greater proteolysis inhibition is commonly associated to a reduced beef quality (Pringle et al., 1997). The ratio between calpain and calpastatin activities determines the potential to tenderize of distinct muscles during aging. There is evidence showing a positive relationship between the inhibitory activity and calpastatin expression (Souza et al., 2019); nevertheless, other did not show the same relationship (Martins et al., 2017) within indicine cattle.

Conclusion

Temperament data had little association to the pH groups. However, cattle with indication of greater handling stress response when facing a new environment (RT1) is related to carcasses with resistant pH decline. Beef of LL from indicine cattle that exhibited a pH between 5.8 and 5.9 at 24 h *pm* showed a similar tenderization rate and extension during 21 days, even though steaks at 14 days in this group could be mostly classified

as tough. However, this group was related to lower cooking loss steaks, evidencing greater water holding capacity. Since greater water holding capacity could be related to a reduced shelf life and potentially affect the appearance of beef *in natura*, these observations need further investigation. The pH incidence within the range represented by the pH–Res group or even higher pH values should be surveyed nationally, as well as consumer perception regarding beef appearance and texture.

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Authors' Contributions

Conceptualization: Ramos, P.M.; Delgado, E.F.; **Data analysis:** Ramos, P.M.; Santos–Donado, P.R; Oliveira, G.M.; Silva, S.L.; Martello, L.S. **Design of methodology:** Ramos, P.M.; Delgado, E.F. **Writing and editing:** Ramos, P.M.; Donado, P.R.S.; Oliveira, G.M.; Contreras–Castillo, C.J.; Scheffler, T.L.; Silva, S.L.; Martello, L.S.; Delgado, E.F.

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