



ANIMAL SCIENCE

Main altered characteristics in the meat of young cattle of different sexual conditions supplemented in tropical pasture

ODILENE DE S. TEIXEIRA, DIEGO S. MACHADO, LUCAS B. PEREIRA, NATHÁLIA P. REIS, CAMILLE C. DOMINGUES, JOHN L. KLEIN, JONATAS CATTELAM, JOSÉ L. NÖRNBERG, DARI C. ALVES FILHO & IVAN L. BRONDANI

Abstract: The aim was to identify the predominant variables in the differentiation of meat quality of cattle submitted to surgical castration, immunocastration, or non-castration and finished in a tropical pasture. Thirty-nine crossbred cattle were used and distributed in three treatments: i) surgical castration; ii) immunocastration; and iii) non-castration, with an initial mean age of 14.06 ± 0.72 months and a mean weight of 284.10 ± 31.40 kg. We used the principal component analysis to differentiate the qualitative meat characteristics between the treatments. Based on that analysis, we found that the first three principal components explained 71.44% of the total variation in the meat quality data, which ensures that the variation found is associated with the effect of the treatments. The characteristics correlated with the first three principal components and responsible for the discrimination between sexual conditions were subcutaneous fat thickness, instrumental meat color, cooking loss and shear force. These characteristics were similar among castrated animals, regardless of the methods. Therefore, immunological castration preserves the attributes of the meat and prevents possible damage to the physical and mental integrity of the animals. Finally, principal component analysis is an important methodology in the objective investigation of beef meat attributes.

Key words: beef cattle, immunocastration, meat quality, multivariate analysis, non-castration, surgical castration.

INTRODUCTION

In beef cattle production, the option of not castrating males is justified by the optimization of biological characteristics related to animal performance. The production of natural hormones, such as testosterone, improves the rate of weight gain and feed efficiency compared to castrated males (Machado et al. 2018). However, the qualitative characteristics of the meat may be disadvantaged since the deposition of fat on the carcass is later (Antonelo et al. 2017, Moreira

et al. 2017) and cause damage to the meat color (Miguel et al. 2014, Gómez et al. 2017).

In this sense, there is a consensus between researchers and producers that castration improves the carcass and meat attributes. However, there is a paradox regarding this practice, which is motivated by the negative perception of pain associated with the surgical castration procedure, reducing animal welfare (Canozzi et al. 2020). Therefore, the growth of population awareness of animal welfare has led to the assessment of standard livestock practices, such as the physical castration of

animals, and the search for alternative methods (Needham et al. 2017). Grandin (2014) reinforces that when cattle breeding occurs in extensive systems, one leading management that can violate the physical and mental integrity of the animals is surgical castration.

Given the above, immunocastration stands out, as this castration method is the least invasive for cattle and regulates sexual function with less impairment of welfare. Through immunocastration, immunization against the gonadotropin-releasing hormone (GnRH) occurs, producing antibodies that neutralize it and occurring the suppression of reproductive hormones (Janett et al. 2012). In other species, such as pigs, immunocastration has already been proven to be an alternative to surgical castration in carcass and meat quality (Gispert et al. 2010). In cattle, the researches focus on the evaluation of animals finished in feedlots and at older ages, between 23 to 27 months (Miguel et al. 2014, Antonelo et al. 2017, Gómez et al. 2017), this being the period in which the characteristics of the meat begin to lose quality. In this context, we hypothesize that immunocastrated cattle finished in pasture maintain the main characteristics desired in the meat while preserving the welfare requirements.

Therefore, through an analytical methodology with recent application in the meat characterization (Baldassini et al. 2017), we propose identifying the predominant variables in the differentiation of meat quality from surgically castrated, immunocastrated, or non-castrated cattle. The principal components analysis is appropriate to elucidate in a set of original variables those that effectively influence the final result in a clear, objective way and eliminate repeated information (Hair et al. 2009).

MATERIALS AND METHODS

Ethical note

All procedures were approved by the Ethics Committee on the Use of Animal (122/2014) from the department of Animal Science.

Experimental design and treatments

The experimental design was completely randomized, with three treatments and the animal as experimental unit ($n = 13$). The treatments evaluated were: non-castrated (control), immunized, and castrated (surgical castration).

Animals, area, experimental period and feed

Thirty-nine Charolais x Nellore crossbred animals of 14.06 ± 0.72 months of age and an initial average weight of 284.10 ± 31.40 kg were used. The experimental area corresponded to 8.04 ha of Aruana grass (*Panicum maximum* Jacq cv. Aruana) divided into 12 paddocks of 0.67 ha each, with four area replications (paddocks) per treatment. The grazing method adopted was continuous with a variable stocking rate (Mott & Lucas 1952) from January to April (summer-autumn).

The diet consisted of Aruana grass and energy supplementation, in a proportion of 1.0 kg dry matter/100 kg live weight. The supplement was provided once a day at 2:00 PM, in a feed trough with 66 cm of feed area/animal. All treatments had unrestricted access to water in drinking fountains and mineral supplementation, consisting of sodium chloride and dicalcium phosphate. The Aruana pasture was managed to maintain the forage mass of around 2600 kg of dry matter/ha. The supplement provided was formulated based on the NRC (2000) with a composition of 62.7% white oat grain, 35.0% ground corn grain, 1.3% calcitic limestone,

and 1.0% urea. The chemical composition of the grains used in the supplement is in Table I. Grazing simulations were carried out during the experimental period for a sampling of forage apparently consumed, according to the technique described by Euclides et al. (1992). The forage samples were forwarded to determine the chemical composition (Table I) after pre-drying in a forced air oven at 55°C until reaching constant weight.

Castration management

The technique used for surgical castration was open orchiectomy by removing the apex of the scrotum, with exposure of the testicles for the section using a scalpel (blade No. 24). Animal containment guidelines were followed according to Resolution No. 928 of November 13 of the CFMV (2009). After verifying the testicles in the scrotum and cleaning with 70% ethyl alcohol, a local anesthetic based on lidocaine hydrochloride (2%) was applied at a dose of 7.0 mL in each spermatic cord as well as an anti-inflammatory, containing an active ingredient flunixin meglumine, in the dosage of 1.1 mg/kg. Additionally, we used an antibiotic with the active ingredient oxytetracycline (20%) in the dosage of 1 mL/10 kg of body weight. Antibiotic and anti-inflammatory were administered

intramuscularly on the day of castration and days 2, 4, and 6 after castration. Also, we applied a healing spray and a repellent at the incision site.

Immunocastration was performed with the application of a bovine immunocastration product (Bopriva[®]; GnRF analog, with carrier protein; Zoetis Indústria de Produtos Veterinários LTDA) in the dosage of 1.0 mL per animal. Immunized steers received three doses of the Bopriva[®] vaccine at 11, 12, and 15 months of age. According to the manufacturer, the bovine will be immunocastrated after two applications of the bovine immunocastration product (dose + reinforcement), with suppression of testosterone between seven and 14 days after the reinforcement. It was recommended that castrations (surgical and immunological) occur concurrently.

Slaughter and carcass measurements

Before the shipment to the slaughterhouse, steers were weighed after fasting for 12 hours of solids and liquids to obtain the slaughter weight. Animals were slaughtered in a commercial abattoir located in Santa Maria, RS, Brazil (30 km away from the research facility), following the slaughterhouse's procedures. The steers were slaughtered with 421.61, 391.29, and 412.82kg, respectively, for castrated, immunized, and

Table I. Bromatological composition of ingredients and grazing simulation of the Aruana pasture in each sexual condition during the experimental period.

Ingredients	Bromatological composition (%)						
	DM ¹	OM	CP	NDF	ADF	EE	IVOM
Oat white	90.20	97.41	13.15	16.44	5.86	6.73	85.42
Corn	88.89	98.41	8.21	10.54	1.87	4.77	97.77
Grazing simulation	DM ¹	OM	CP	NDF	ADF	EE	TDN
Non-castrated	27.16	89.65	14.90	72.22	38.99	1.97	53.44
Immunized	26.82	89.37	14.76	72.11	39.09	2.00	53.27
Castrated	27.81	89.40	14.33	72.07	40.12	1.92	52.62

¹Percentage of natural matter; DM = Dry matter; OM = Organic matter; CP = Crude protein; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; EE = Ether extract; IVOM = *In vitro* digestibility of organic matter.

non-castrated (Teixeira et al. 2018). The pH 24 hours index was measured on the *Longissimus thoracis* (LT) muscle at the 12th rib, using a digital pH measurement instrument (Testo 205[®]) after 24 hours of cooling the carcasses.

After measuring the metric characteristics, the right half-carcass was sectioned at the height of the 12th rib, aiming to expose the LT, to trace its contour in tracing paper, which was later determined, using a digital scanning table and the Inkscape[®] software to determine the area rib eye area. The subcutaneous fat thickness (SFT) was measured at the same site, using a digital pachymeter, as the average of three observations. After dissection, the LT muscle portion was vacuum packed and frozen at -18°C for further determine meat quality characteristics.

Meat quality

After 30 minutes of exposure to air, the meat color was measured in the *Longissimus thoracis*. The parameters evaluated were: brightness (L*), redness (a*), and yellowness (b*), which were assessed by the CIE L* a* b* color system using a colorimeter (Minolta Chroma Meter CR-300, Osaka, Japan). At this moment, the degree of the meat marbling was also subjectively determined, according to Müller (1987).

One 2.5 cm thick slice was removed from the frozen LT muscle. The slice was weighed while still frozen and after thawed for 24 hours. The temperature was kept between 2 and 5°C to determine the loss of liquid during the thawing process. In an electric oven, it was then cooked to an internal temperature of 71°C (monitored with a thermometer) to evaluate the loss of liquid during cooking. It was measured the final weight, and after cooling the beef, removed the cylindrical samples. Six cylindrical portions of the slice, cut in the longitudinal direction of the fiber (1.0 cm diameter), were removed

from each sample using a steel cutter with a cylindrical mold to measure the shear force. The Warner-Bratzler shear force measurement was performed using a texture analyzer (Stable Micro Systems, TA.XT plus Texture Analyzer, UK), and the test speed was 3.30 mm/s.

Statistical analysis

Data collected were submitted to multivariate analysis of variance (MANOVA), using the mathematical model following: $Y_{ijk} = \mu_k + SC_{ik} + \epsilon_{ijk}$, in which: Y_{ijk} = observed value of the k -th variable, under the i -th sexual condition of the j -th replicate; μ_k = overall mean of the k -th variable; SC_{ik} = effect of i -th sexual condition; ϵ_{ijk} = random effects associated to observation Y_{ijk} assuming a normal distribution, independent and identically distributed – NIID ($0, \sigma^2$); $\forall i = 1, 2, 3$; $\forall j = 1, 2, \dots, 13$ and $\forall k = 1, 2, \dots, 9$. We used the Wilks (2006) test the hypothesis of equality of the vectors of the sexual condition effects.

Subsequently, the principal components analysis (PCA) was performed, utilizing the standardized means of meat quality characteristics and the correlation matrix (R). The principal component technique consists of transforming a set of variables $X_1, X_2, X_3, \dots, X_n$, representing an interdependence structure, into a set of variables Y_1, Y_2, \dots, Y_n , where n is the number of variables. The Y_{js} are linear functions of the X_{is} , independent of each other. Their variances are ordered so that it is possible to compare the sexual conditions of the steers using only those that present greater variance. Y_j is a principal component, given by Y_j (or PC_j) = $a_{1j}X_1 + a_{2j}X_2 + \dots + a_{nj}X_n$. The number of principal components was chosen based on the Kaiser (1960) criteria, in which only the principal components with eigenvalues greater than 1 explained the most important part of the variation between sexual conditions.

All statistical analyses were performed in R Core Team (2017) software, using the *matcor* and *prcomp* functions of the *Stats* package.

RESULTS

The multivariate analysis of variance was significant by the *Wilks-Lambda* test ($\Lambda = 0.0236$), so we performed the principal component analysis (PCA). According to Kaiser's criterion (Kaiser 1960), the first three principal components must be studied (eigenvalue > 1), as they contain the most important variations. In this sense, we found that of the nine original dimensions (i.e., values measured for each characteristic studied), the first three PCs explained 71.44% of the total variation in meat quality data of male cattle in different conditions (Table II). It ensures that much of the variation found is associated with the effect of treatments.

The characteristics correlated with the first three principal components (PC1, PC2, and PC3) and responsible for the discrimination between sexual conditions are subcutaneous fat thickness, instrumental meat color (L^* , a^* , and b^* values), cooking loss, and shear force (Table III). The characteristics pH 24 hours *post*

mortem, marbling, and thawing loss were similar between the sexual conditions evaluated.

The principal component 1 (PC1) was more closely associated with subcutaneous fat thickness and meat color. However, the linear correlations were negative between PC1 and all variables (Table III). When considered PC2, the cooking loss showed greater discriminatory power, correlating positively. For PC3, the greatest discriminatory power occurred for shear force with a correlation of -0.65.

Table IV shows the descriptive statistics of beef quality characteristics of cattle correlated with the first three PCs. These characteristics are responsible for the greatest variability in response to immunocastration, surgical castration, or non-castration.

The characteristics correlated with PC1 (SFT , L^* , a^* , and b^*) also varied significantly between each other, with values ranging from 0.49 to 0.89 (Table V). However, none of the variables mentioned above correlated with the cooking loss and the shear force, which were correlated with each other ($r = 0.44$).

After identifying that PC1 represented 37.74% of data total variation and that PC2 represented 19.44%, the vectors of the characteristics

Table II. Eigenvalues, proportion of total variance explained, and cumulative proportion of each principal component (PC) considering nine qualitative meat characteristics.

Component	Eigenvalue (λ)	Proportion of variance (%)	Accumulated proportion (%)
PC1	1.8430	37.74	37.74
PC2	1.3226	19.44	57.18
PC3	1.1329	14.26	71.44
PC4	0.9317	9.64	81.08
PC5	0.8161	7.40	88.48
PC6	0.6723	5.02	93.51
PC7	0.6215	4.29	97.80
PC8	0.3992	1.77	99.57
PC9	0.1968	0.43	100.00

that varied between sexual conditions were expressed in Figure 1. Since non-castrated cattle produced meat with greater loss of fluids at cooking, castrated by the surgical method stood out for the higher values of color a^* and b^* . Castrated animals showed superiority in the subcutaneous fat thickness and brightness (L^*) regardless of the method, while non-castrated animals had their carcasses depreciated in these aspects (Figure 1). The shear force, although correlated to PC3, was superior in the meat of immunized steers, even without a relevant discrepancy between the values: 5.16, 5.26, and 5.08 kgF/cm³ for non-castrated, immunized, and surgically castrated, respectively, (Table IV).

DISCUSSION

The analysis related to the meat quality of cattle in the current study showed that the subcutaneous fat thickness, the instrumental meat color (L^* , a^* , and b^* values), and the cooking loss and shear force are the main characteristics that differentiate between the

sexual conditions studied. This finding was predicted by the principal component analysis, which has among its principles to suggest the disposal of redundant or non-discriminatory variables (Jerez-Timaure et al. 2013, Giaretta et al. 2018).

In this sense, the variables related to marbling, pH, and loss of liquids on thawing were discarded from the present study, because they are not discriminatory between the sexual conditions evaluated. Thus, future studies can reduce the number of variables collected, facilitating data collection operationalization without losing information and analytical work reliability. However, it is noteworthy that although marbling fat has not been a discriminating variable between sexual conditions, we do not recommend its exclusion, based on the data from this study. This is because the animals in this study were slaughtered in a light weight and, therefore, it is suggested that this fat did not get enough time to develop. The fat deposition rate in the organism of the animal follows an order, according to the advance of maturity,

Table III. Description of the variables included in the study and correlation values with the principal components 1, 2 and 3 (PC1, PC2 and PC3).

Variables	Correlation ¹		
	PC1	PC2	PC3
pH 24 hours	0.3856	-0.5267	-0.1025
Subcutaneous fat thickness (mm)	-0.8749*	-0.0439	-0.0890
Marbling ²	-0.3095	-0.5822	-0.5369
Brightness (L^*)	-0.7739*	-0.1095	0.1487
Redness index (a^*)	-0.8469*	0.3607	-0.0370
Yellowness index (b^*)	-0.9475*	0.1967	0.0693
Thawing loss (%)	0.3066	0.5246	0.5345
Cooking loss (%)	0.2260	0.6735*	-0.4870
Shear force (kgF/cm ³)	0.1672	-0.4704	-0.6524*

¹Correlations considered in the interpretation of the principal components analysis ($r > 0.60$).

²Scale of 1 to 18 points, being: 1-3 trace; 4-6 light; 7-9 small; 10-12 medium; 13-15 moderate; 16-18 abundant. * $P < 0.05$.

the development of visceral, intermuscular, subcutaneous, and lastly, intramuscular (marbling) fat occurs (Owens et al. 1993).

Among the non-discriminatory variables, the pH values are the most surprising, because as presented in other studies, it was assumed that lower values for the final pH of the meat were found in surgically castrated and/or immunocastrated males when compared to non-castrated (Miguel et al. 2014, Antonelo et al. 2017, Gómez et al. 2017). The variation found by these authors may be related to the more reactive temperament in the pre-slaughter of non-castrated animals since the greatest stress determines a higher pH, which was also evidenced in the present research. When assessing agonistic behavior, we observe that the non-castrated individuals presented a greater number of activities, such as threats and fights, concerning the immunocastrated ones during the termination period (Teixeira et al. 2018). Thus, we assume that the youngest age of the animals (18 months), concerning the studies mentioned above, has influenced this difference not to be pronounced. It should be noted that regardless of the treatment, the meat presented pH values in the range considered ideal (Muchenje et al. 2009), with values of 5.5,

5.6, and 5.6 for castrated, immunized, and non-castrated, respectively.

We observed in our study the association of sets of characteristics with different principal components. The principal component 1 refers to the deposition of subcutaneous fat and the meat color, PC2 to cooking loss, and PC3 to shear force. These associations are also reported in the literature. According to Jerez-Timaure et al. (2013), the first PC grouped variables related to meat tenderness, the second PC to weight and carcass finishing, and the third PC to skeletal maturity of Brahman-cross cattle. In turn, Baldassini et al. (2017) observed that the meat color (yellow intensity and chromaticity) defined the first PC. In meanwhile, the second PC indicated meat with greater variation in tenderness (shear force) and tenacity (cooking losses), and the third PC was related to fat deposition (subcutaneous and intramuscular).

Subcutaneous fat thickness was lower for non-castrated cattle, as verified by Amatayakul-Chantler et al. (2012) and Moreira et al. (2017). This result is attributed to the anabolic testosterone effect, which potentiates protein deposition to the detriment of fat deposition in the carcass (Machado et al. 2018). It is noteworthy that only the non-castrated animals did not reach the

Table IV. Descriptive statistics of the characteristics associated with the first three principal components by the criteria adopted in this study.

Variables	Non-castrated		Immunized		Castrated	
	Mean±SD	Min – max	Mean±SD	Min – max	Mean±SD	Min – max
Subcutaneous fat thickness (mm)	2.57±0.46	2.00 – 3.66	3.52±1.61	2.33 – 6.66	3.73±1.63	2.33 – 8.00
Brightness (L*)	37.02±2.04	34.03 – 39.87	37.24±2.82	31.55 – 40.77	37.80±2.04	28.28 – 43.56
Redness index (a*)	13.60±1.08	11.03 – 15.06	15.16±2.50	12.11 – 21.15	15.47±1.77	13.01 – 19.38
Yellowness index (b*)	5.33±0.73	4.16 – 6.80	6.06±1.39	4.28 – 8.92	6.50±1.02	4.82 – 8.66
Cooking loss (%)	27.83±3.21	18.77 – 32.03	26.02±3.48	17.22 – 29.30	27.06±2.12	22.92 – 29.90
Shear force (kgF/cm ³)	5.16±0.79	3.43 – 5.99	5.26±1.19	3.25 – 7.77	5.08±1.25	3.51 – 6.92

minimum fat thickness required by the Brazilian refrigeration industry of 3.0 mm. Amatayakul-Chantler et al. (2013) and Machado et al. (2018) also report the similarity for SFT between those immunized and castrated by the surgical method. However, the period of action of the vaccine may influence this characteristic. Moreira et al. (2017) evaluated steers finished on tropical pasture and obtained lower SFT values for immunocastrated cattle (with 153 days between the final dose and slaughter) concerning surgically castrated. Therefore, there was a feed condition similar to the one in this study, but with a shorter period of action of the Bopriva[®] vaccine (210 days).

However, although the SFT was below the threshold value reported to protect the carcass during cooling, it did not interfere the meat tenderness, as shown by shear force correlation, however it affected the meat color. According to Miguel et al. (2014), non-castrated male cattle tend to have a less fat thickness in the carcass, and, consequently, there is a harmful effect on the meat color. The values of L*, a*, and b* were higher for meat from surgically castrated cattle, although the first coordinate mentioned had an intermediate result for the surgically castrated and immunized cattle.

Meat from castrated steers (surgically or immunized) was redder compared to non-castrated steers. The a* and b* values found in

the current study showed that castration can be used to improve the meat color. The values found agreeing with other studies (Miguel et al. 2014, Antonelo et al. 2017, Gómez et al. 2017), which also showed lower a* and b* values in meat of non-castrated animals. It is an important characteristic of products that are commercialized since the physical appearance of the meat cuts interferes with acceptance, purchase decisions, and consumer satisfaction (Muchenje et al. 2009), once values of a* above 14.5 are more acceptable by consumers from different continents (Holman et al. 2017).

The cooking loss was higher in meat from non-castrated compared to castrated animals, regardless of the method. According to Baldassini et al. (2017), beef samples that had higher cooking losses also showed lower backfat thickness (BFT) values. Thus, in the current study, these cooking loss results could be related to BFT, since the non-castrated group presented lower fat thickness compared to castrated animals. In addition, Amatayakul-Chantler et al. (2013) observed similar cooking loss results in meat of *Bos taurus indicus* animals submitted to surgical or immunization castration.

Concerning PCA, Baldassini et al. (2017) found that cooking losses and shear force were associated with the same main component (PC2). This component would discriminate animals

Table V. Correlation matrix between six variables associated with the first three principal components.

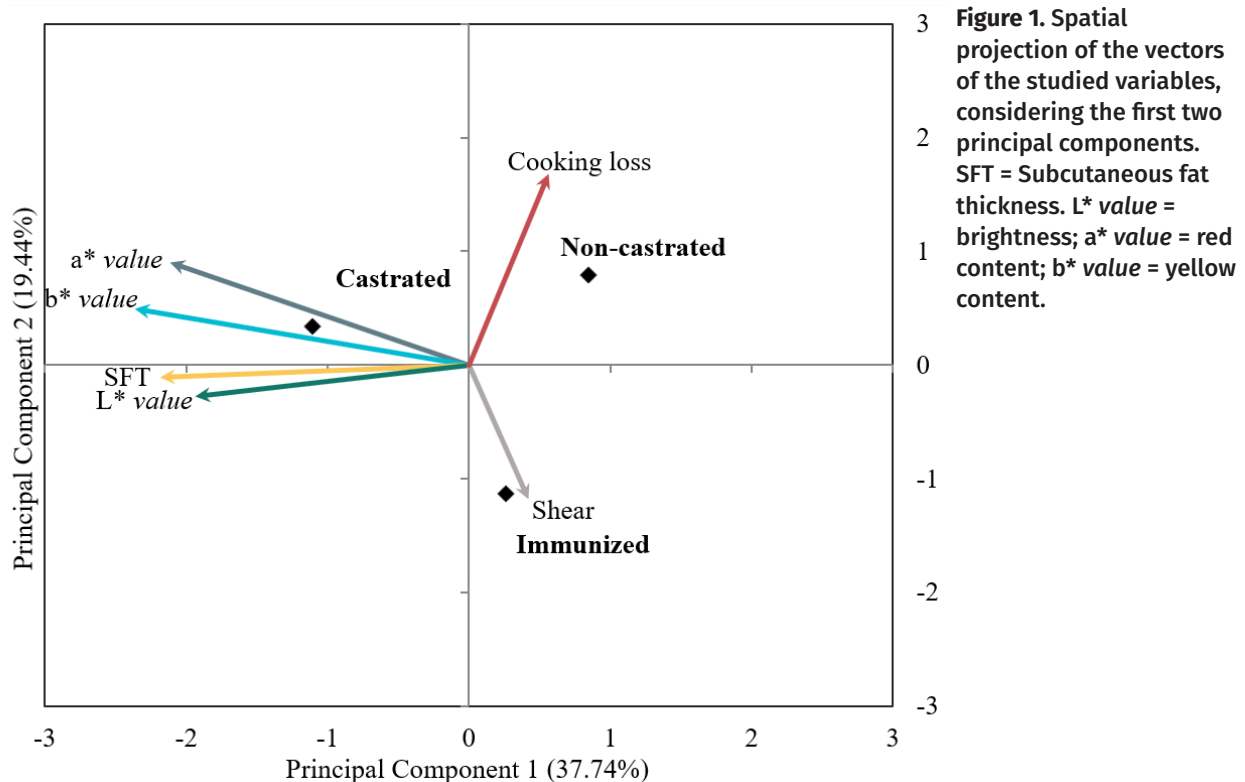
Variables	L*	a*	b*	Cooking loss	Shear force
SFT	0.5471**	0.7224***	0.7593***	-0.1546 ^{ns}	-0.1421 ^{ns}
L*		0.4905**	0.7477***	-0.2827 ^{ns}	-0.1474 ^{ns}
a*			0.8956***	0.0190 ^{ns}	0.0685 ^{ns}
b*				-0.1265 ^{ns}	-0.0743 ^{ns}
Cooking loss					0.4373 [*]

SFT = Subcutaneous fat thickness; L* = brightness; a* = red content; b* = yellow content. *P < 0.05; **P < 0.01; ***P < 0.001; ^{ns} = no significant.

according to these characteristics, a result not observed in the present study, as shear force was associated with PC3. However, the data of this research corroborate with Giaretta et al. (2018), who found that cooking losses were related to the third PC, while shear force was correlated to the second PC, i.e., principal components that separately discriminate such characteristics. Losses during meat cooking influence the yield of the product in food preparation. Also, Modzelewska-Kapitula et al. (2012) reiterate that these losses affect the palatability attributes and influence the acceptability of meat cuts.

Shear force values were the only ones to differentiate sexual conditions in PC3, but with a proportion of the variance explained by this PC of only 14.26%. The highest values of shear force comprise the meat of immunized animals 5.26 kgF/cm³ and the lowest ones surgically castrated 5.08 kgF/cm³, while the non-castrated ones presented 5.16 kgF/cm³. These values classify all

groups in the same class of instrumental meat tenderness, next to the tender meat. Similar shear values are reported in meat from animals raised in an extensive system, which submitted to surgical or immunological castration (Amatayakul-Chantler et al. 2013, Machado et al. 2019). When comparing castration methods to non-castration, there are reports of similarities in meat tenderness (Machado et al. 2018, Miguel et al. 2014). Amatayakul-Chantler et al. (2012) found less tender meat for non-castrated animals compared to castrated animals. Yet, Gómez et al. (2017) found less tenderness in the meat of surgically castrated steers than non-castrated steers. The data reported on softness are contradictory among the research cited. This inconsistency can be related to several factors, such as the breed, age, and temperament of the animal, as well as slaughter handling, the treatment of carcasses in the refrigerator, and



the procedures involved in preparing meat during cooking.

In short, the sexual condition of the animals was responsible for important changes in the meat characteristics. In this sense, with a view to the ideal fat finish and an improvement in the color and meat tenderness, it is suggested using immune castration, since it is recognized as a less invasive method to animals, favoring the mitigation of accident risks both among animals as with their handlers. Finally, it is noteworthy that even when castration is performed at prepubertal age, immunocastration is the best option in relation to the surgical procedure, even if this implies in more than two vaccine applications until the slaughter of the animal (Machado et al. 2019). In addition, the analysis of principal components is an important methodology in the objective investigation of beef attributes. It allows the selection of characteristics with the greatest impact, which reduces the dimensionality of the data.

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ODILENE DE S. TEIXEIRA¹

<https://orcid.org/0000-0002-6961-7100>

DIEGO S. MACHADO²

<https://orcid.org/0000-0002-2406-280X>

LUCAS B. PEREIRA¹

<https://orcid.org/0000-0003-3627-724X>

NATHÁLIA P. REIS¹

<https://orcid.org/0000-0002-6721-0833>

CAMILLE C. DOMINGUES¹

<https://orcid.org/0000-0003-1781-7626>

JOHN L. KLEIN¹

<https://orcid.org/0000-0001-8337-4152>

JONATAS CATTELAM³

<https://orcid.org/0000-0002-4395-2189>

JOSÉ L. NÖRNBERG⁴

<https://orcid.org/0000-0002-8366-4480>

DARI C. ALVES FILHO¹

<https://orcid.org/0000-0003-2559-7504>

IVAN L. BRONDANI¹

<https://orcid.org/0000-0002-6526-3042>

¹Programa de Pós-Graduação em Zootecnia, Universidade Federal de Santa Maria, Departamento de Zootecnia, Campus Sede, Avenida Roraima, 1000, Cidade Universitária, Camobi, 97105-900 Santa Maria, RS, Brazil

²Instituto Federal de Educação, Ciência e Tecnologia Farroupilha, Campus Alegrete, RS-377, Km 27, Passo Novo, 97555-000 Alegrete, RS, Brazil

³Programa de Pós-Graduação em Saúde, Bem-Estar e Produção Animal Sustentável na Fronteira Sul, Universidade Federal da Fronteira do Sul, Campus de Realeza, Avenida Edmundo Gaievski, 1000, Rodovia BR-182, Km 466, 85770-000 Realeza, PR, Brazil

⁴Universidade Federal de Santa Maria, Departamento de Tecnologia e Ciência dos Alimentos, Campus Sede, Avenida Roraima, 1000, Cidade Universitária, Camobi, 97105-900 Santa Maria, RS, Brazil

Correspondence to: **Odilene de Souza Teixeira**

E-mail: odilene_rs@hotmail.com

Author contributions

Odilene de Souza Teixeira, Diego Soares Machado, Dari Celestino Alves Filho and Ivan Luiz Brondani: conceptualization, investigation, methodology, formal analysis, writing-preparation, writing-review and editing. Lucas Braido Pereira, Camille Carijo Domingues, John Lenon Klein and Nathália Pasi Reis: methodology, data collection, writing-review and editing. Jonatas Cattelan: supervision, data collection, formal analysis, writing-preparation. José Laerte Nörnberg: methodology, data collection, writing-preparation.

