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Author	Bismarck Moreira Santiago[1], Welder Angelo Baldassini[1,2*], Laís de Aquino Tomaz[1], Leone Campos Rocha[1], Wellington Bizarria dos Santos[2], Rogério Abdallah Curi[1,2], Luis Artur Loyola Chardulo[1,2], Otávio Rodrigues Machado Neto[1,2]
Affiliation	1 School of Agricultural and Veterinarian Sciences, São Paulo State University (FCAV UNESP), Jaboticabal, São Paulo, Brazil. **2** School of Veterinary Medicine and Animal Science, São Paulo State University (FMVZ UNESP), Botucatu, São Paulo, Brazil.
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ORCID (All authors must have ORCID) https://orcid.org	Bismarck Moreira Santiago https://orcid.org/0000-0002-2479-9000 Welder Angelo Baldassini https://orcid.org/0000-0003-0840-2082 Laís de Aquino Tomaz https://orcid.org/0000-0001-8044-1604 Leone Campos Rocha https://orcid.org/0000-0003-3715-0312 Wellington Bizarria dos Santos https://orcid.org/0000-0001-5984-533X Rogério Abdallah Curi https://orcid.org/0000-0001-6289-0406 Luis Artur Loyola Chardulo https://orcid.org/0000-0003-2656-1562 Otávio Rodrigues Machado Neto https://orcid.org/0000-0002-4449-7771
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CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Welder Angelo Baldassini

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Email address – this is where your proofs will be sent	w.baldassini@unesp.br
Secondary Email address	welder.ab@alumni.usp.br
Postal address	Rua Prof. Dr. Walter Maurício Correa, s/nº Bairro: Unesp - Campus de Botucatu CEP: 18.618-681 - Botucatu, SP. Caixa Postal: 560
Cell phone number	+55 19 97143 9383
Office phone number	+55 14 3880 2989
Fax number	N/A

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Title

Comparison of dental carcass maturity in non-castrated male F1 Angus-Nellore cattle finished in feedlot

Abstract

Dental classification of carcasses is used as a parameter of cattle maturity at slaughter, and it can influence carcass and meat quality traits. Brazilian beef-packing companies use the number of permanent incisor (P.I.) teeth as a parameter for bonus and certification of carcasses with superior quality. However, when non-castrated male such as F1 Angus-Nellore (*Bos taurus* x *Bos* indicus) are slaughtered, only animals without P.I. teeth are subsidized by the breed association. We evaluated these animals finished in feedlot for 180 days with zero versus two P.I. teeth on the carcass and meat quality traits. At the time of slaughter, 88 carcasses were selected, forming two treatments according to dental carcass maturity (0 versus 2 P.I. teeth; 44 animals per category). It was demonstrated that the number of P.I. teeth (0 versus 2 P.I.) did not influence ($P > 0.05$) carcass (weights, yield, cooling loss, ribeye area and the backfat thickness) and meat quality traits (*Longissimus thoracis* chemical composition, color, cooking losses, shear force and pH). Thus, dental carcass maturity (zero versus two P.I. teeth) does not influence non-castrated male F1 Angus-Nellore finished in feedlot for 180 days. This is the first study to demonstrate that carcasses of non-castrated male F1 Angus-Nellore with two P.I. teeth should be subsidized in a similar way to those with zero P.I. teeth. Moreover, Brazilian beef-packing companies could produce heavier and leaner carcasses of acceptable quality though the use of crossbred cattle such as non-castrated F1 Angus Nellore.

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Keywords: Beef cattle; Bos indicus; dentition; meat quality; tenderness.

Introduction

In beef cattle, there are differences between *Bos indicus* (e.g. Brahman and Nellore) and *Bos taurus* (e.g. Simental and Charolais) breeds in the eruption age of permanent incisor (P.I.) teeth. The loss of primary teeth in taurine occurs earlier than in zebu. Thus, changes from primary incisor to P.I. teeth may occur between 18 and 28 months in taurine, while in zebu it occurs between 20 and 24 months (Gomide et al., 2009). The chronological age and dentition effects on carcass and meat quality have been studied by researchers in South Africa (Moholisa et al., 2017), Australia (Wythes and Shorthose, 1991), United States (Lawrence et al., 2001) and Brazil (Duarte et al., 2011).

Approximately 80% of the beef cattle herd in Brazil belongs to *Bos indicus*, and the Nellore breed is the most adopted due to its adaptability to the tropical climate (Ferraz and Felício, 2010). However, the need for increased productivity and the demand for improved meat quality by consumers has led beef cattle producers to adopt cross-breeding with European breeds (*Bos taurus*), mainly Aberdeen Angus, generating F1 Angus-Nellore to obtain better performance, carcass traits and meat quality when compared to pure zebu animals (Miguel et al., 2014). In the tropical regions of Brazil, it is common to use non-castrated animals (bulls) of advanced maturity in the finishing farms. It can compromise the meat quality, affecting characteristics such as color, marbling and tenderness. As indicated by a survey, 95% of the animals finished in Brazilian feedlots are males, 73% from these are Nellore, followed by 22% of crossbred animals and 5% of other genotypes (Costa Junior et al., 2013).

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Only two studies have evaluated the dental classification of carcasses of Nellore at slaughter and its relation to the meat quality of animals finished in tropical pastures, whereby carcass and meat traits of bulls (Duarte et al., 2011) and steers (Pflanzer and Felício, 2009) were described. However, animals with zero versus two P.I. teeth were not compared in these two studies.

Some cattle breeding associations recommended that carcasses of non-castrated male animals should have only primary teeth to be subsidized, i.e., without P.I. teeth. However, it is considered that the age difference assessed by dental carcass maturity between zero and two P.I. teeth is small and not enough to affect the meat quality of animals, especially when finished in feedlot for more than 160 days. In the literature, there are no studies that have evaluated this hypothesis using this biological model.

In this context, the aim of this study is to evaluate the effect of dental maturity (zero and two P.I. teeth) on the carcass traits and meat quality of non-castrated male F1 Angus-Nellore cattle finished in feedlot.

Material and Methods

Animals and diet

All the procedures performed in the experiment were approved by the Ethics Committee on Animal Use of the College of Veterinary Medicine and Animal Science - UNESP (CEUA protocol no. 07595/2019).

The animals originated from the experimental feedlot belonging to "Fazenda Turbilhão", in the city of Estrela D'Oeste-SP, Brazil. In the feedlot, 640 non-castrated male F1 Angus-Nellore cattle were submitted to a diet formulated (Supplementary Material, Table S1) to meet the maintenance and weight gain requirements of 1.5 kg/day according to the NRBC (2016). The composition of the diets was obtained by

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feed analysis (AOAC, 2005), followed by the procedures of determination of DM (method 976.05), CP (method 976.05, N * 6.25) and ash content (method 942.05). For NDF analysis, samples were treated with alpha amylase at a stable temperature without the addition of sodium sulfite and corrected for ash (Mertens, 2002). The EE analysis was conducted by the Soxhlet extraction (method 920.39). The animals were allocated in collective pens, equipped with a bunk and an automatic drinking trough, for an experimental period of 180 days. The total diet was provided twice a day at 08:30 AM and 03:30 PM.

Slaughter and carcasses selection

After the experimental period of 180 days and 16-hour fasting, all animals were slaughtered in a commercial slaughterhouse (Estrela D'Oeste, SP, Brazil) on the same day following the normal procedures of federal inspection. At this moment, among the 640 slaughtered animals, following head inspection, the number of permanent incisors (P.I.) was recorded for each animal. Subsequently, after the slaughter data had been collected, 44 carcasses were randomly selected per dentition group, totaling 88 carcasses grouped in two categories according to the number of P.I. (zero [n= 44] and two [n=44] P.I. teeth). Due to the random selection of animals (carcasses) at the slaughterhouse, the initial body weight was 282.24 kg for zero P.I. and 291.88 kg for two P.I. (P < 0.001). Therefore, comparison of carcass and meat quality traits was made between animals of different ages, which shows different dental carcass maturity at the slaughterhouse.

Carcass traits

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The hot carcass weight (HCW) was recorded immediately after slaughter and used to calculate the carcass yield. The hot carcass yield was obtained by the formula: $HCY = (HCW/fBW) * 100$, where fBW was the final body weight (before the slaughter).

After 24 hours of chilling (0 - 2° C) the cold carcass weight and cooling losses were recorded. In the right half carcass between the 12th and 13th thoracic vertebrae, the ribeye area (REA) of the *Longissimus thoracis* (LT) muscle and the backfat thickness were evaluated. The REA of LT muscle, between the 12th and 13th thoracic vertebrae, was recorded in transparent plastic before boning and subsequently digitalized and analyzed with the aid of Image J (National Institutes of Health, Maryland, EUA). The backfat thickness (BFT) was determined in the LT muscle using a digital caliper.

A portion of approximately 15 cm in length of the LT was removed from the left 13th rib in cranial direction which, after being identified and individually vacuum packed, was transported to the laboratory. Subsequently, with the help of a band saw, the samples of LT were sectioned into 3 standard steaks of 2.54 cm thickness for analysis of chemical composition, cooking loss, shear force and instrumental evaluation of color. The steaks were again sealed in vacuum bags (polyamide/polyethylene bags) for high vacuum and low oxygen permeability and kept frozen at -20 ℃ until the time of analysis.

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Meat color and pH

The beef samples were thawed at 4 ℃ for 24 hours and exposed to oxygen for 30 minutes at 4 ℃ (blooming time). First, the meat pH was measured using a Hanna digital pH meter (Model HI 99163, Hanna Instruments, Woonsocket, RI) with penetration probe. The pH meter was calibrated using standard pH 4.0 and 7.0 buffers. In the same steak, meat color (L * = luminosity, a * = red intensity, b * = yellow intensity) was measured using the CIELab system of the CR-400 colorimeter (light source A, absorbance angle 10, Y, 0.01 at 160.00% reflectance, Konica Minolta Sensing, Inc., Tokyo, Japan), following the procedures previously described (Baldassini et al., 2017). The colorimeter was calibrated using a standard black and white plate and then three color readings were performed on the surface of the LT muscle sample. An average of the three measurements was generated for each variable (L *, a * and b *). Chroma colorimetric indexes (color saturation) were calculated by the formula $[(a^*)^2 + (b^*)^2]^{0,5}$ and the hue angle (H°) $[\tan^{-1} (b^* / a^*)]$, as described by Cañeque et al. (2004).

Shear force and cooking losses

The samples were placed in a grid over a glass refractory and weighed. Afterwards, a thermocouple was inserted into the geometric center of the samples, coupled to a digital thermometer model DT-612 (ATP Instrumentation, Ashby-de-la-Zouch, England) to monitor the internal temperature of the samples. The steaks were grilled in a preheated oven (Feri90 Venâcio Aires, Rio Grande do Sul, Brazil) equipped with a thermostat to avoid temperature variation. When the internal temperature of the steak reached 40 ℃ the sample was turned and remained in the oven until reaching 71 ℃ internal temperature, according to the methodology described by Wheeler et al. (1996). Then, the samples were kept at room temperature for 15 minutes, weighed and refrigerated at 4 ℃ for 24 hours.

The cooking loss was determined by the weight difference before and after cooking. The cooking losses were measured from drip and evaporation losses. After

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cooling, eight cylinders with 1.27 cm diameter were removed from the parallel direction of the muscle fiber using a hollow punch coupled to an industrial drill. The cylinders were sectioned in Brookfield CT-3 Texture Analyzer (AMETEK Brookfield, Middleborough, EUA) equipment. The results were presented in kilograms (kg) and eight replicate measurements per steak were performed to increase results accuracy.

Chemical composition

The samples were thawed at 4 °C for 24 h and the subcutaneous fat was removed from the LT muscle with the aid of a scalpel, then the steak was ground and homogenized for 5 minutes using a mixer, taking approximately 180 g of sample (Anderson, 2007). Three readings per sample were carried out using a FoodScan LabTM (Foss NIRSystems, Inc., USA). Samples were homogenized again and placed in the plate for the next reading. An average was obtained for the values of moisture, protein, fat and ash and the values were expressed as percentage.

Statistical analysis

Data were tested for distribution and normality of errors and analyzed using the UNIVARIATE and GLM procedures of SAS (2015) version 9.4. (SAS Institute, Inc. University Edition). The animal was considered an experimental unit and dental carcass maturity (treatment) was used as a fixed effect, being tested by analysis of variance (ANOVA), with significance considered at $P \leq 0.05$. Due to the difference found in the initial body weight, this variable was adopted as covariable for the variables of carcass traits, as follow:

$$Y_{ijk} = \mu + p_i + t_i + \varepsilon_{ijk}$$

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Where: Y_{ijk} = the observed value for the response variable obtained for the i^{th} treatment on its j^{th} repetition; μ = the mean of all possible values of the response variable (initial body weight); t_i = the effect of treatment (dental maturity) *i* on the observed value Y_{ijk}; ε_{ijk}= the experimental error associated with the observed value for the response variable Y_{ijk}.

For the variables of meat quality only the fixed effect of dental maturity was used, following statistical model:

$$Y_{ijk} = \mu + t_i + \varepsilon_{ijk}$$

Where: Y_{ijk}= the observed value for the response variable obtained for the i^{th} treatment on its j^{th} repetition; μ = the mean of all possible values of the response variable; t_i = the effect of treatment (dental maturity) *i* on the observed value Y_{ijk}; ε_{ijk} = the experimental error associated with the observed value for the response variable Y_{ijk}.

Results and Discussion

Animals with zero versus two P.I. were similar (P > 0.05) on final body weight (fBW = 578.84 versus 567.75 ± 5.03 kg; 0 versus 2 P.I. teeth, respectively). Additionally, the number of incisor teeth did not influence (P > 0.05) the carcass traits (Figure 1). Thus, no difference was observed for carcass weights, carcass yield, carcass cooling loss, REA and BFT. The experimental groups were similar on meat quality traits (color, cooking loss, tenderness and pH) evaluated in the LT muscle (Table 1), as well as no differences on chemical composition (P > 0.05) of beef samples were observed among 0 versus 2 P.I. teeth. Opposite results were reported in the literature, whereby

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Our study showed that the number of P.I. teeth did not influence ($P > 0.05$) the carcass traits and meat quality (chemical composition, color, cooling losses, tenderness and pH) of Angus-Nellore young bulls. The results indicated that the difference in maturity at slaughter between zero and two P.I. teeth was not enough to affect the meat quality of animals finished in feedlot for more than 160 days. In the literature, there are no studies that have tested this hypothesis using this biological model.

Using zebu animals, carcass dental maturity at slaughter and its relation to the meat quality of animals finished in tropical pastures were described (Pflanzer and Felício, 2009; Duarte et al., 2011). As indicated, carcass and meat traits of non-castrated (Duarte et al., 2011) and castrated male Nellore (Pflanzer and Felício, 2009) were evaluated. In the study by Pflanzer and Felício (2009), the authors used 60 animals and reported that the differences in objective and sensory tenderness of castrated Nellore cattle were an effect of the finishing degree of the carcasses and not due to chronological age or teeth maturity (measured as number of 2, 4 or 6 P.I. teeth). Additionally, Duarte et al. (2011) used 63 non-castrated Nellore cattle and reported that there were no differences in meat tenderness of animals with two (SF = 4.52 ± 0.60 kg) or four (SF = 4.56 ± 0.33) P.I. teeth.

These results confirm the findings of our study and suggest that dental carcass maturity is not a reliable parameter to be associated with carcass and meat quality, specifically tenderness, color and marbling. Similarly, a study confirmed that age assessed by dentition could distinguish differences in tenderness between young grain-fed and older grass-fed carcasses, but not between grass-fed carcasses of different age classes (2 versus 3-6 P.I.) (Moholisa et al., 2017). In literature, additional studies have also shown that carcass classification or grading based on dentition is inadequate to describe variation in beef quality (Strydom, 2011; Acheson et al., 2014).

However, the classification of bovine carcasses in Brazil is mainly performed by the subjective evaluations of maturity (dentition of animals), conformation and finishing, allied to the objective evaluations of gender and hot carcass weight (Sainz and Araujo, 2001). This type of evaluation correlates the eruption of P.I. teeth with animal age, both for zebu and taurine (Kirton, 1989). This classification, dated from approximately three decades, has its limitations as a parameter for the discrimination and determination of superior carcasses in quality within the slaughterhouses, starting with the minimum carcass weights currently required by the main domestic and export markets, which signal for larger animals and finishing, with hot carcass weights above 250 kg (MAPA, 1989). The constant search for superior products in yield and carcass quality has provided remarkable advances in the characterization of earlier animals in muscle growth and finishing, bringing together the classification systems of traditionally exporter countries of better-quality products such as the United States and Australia (Ferraz and Felício, 2010).

A classical study from United States evaluated 200 taurine carcasses and groups with different dental carcass maturity (0, 2, 4, 6 and 8 P.I. teeth; 40 animals per dentition group) and compared castrated male and female cattle (Lawrence et al., 2001). The authors reported that no differences were found for SF, sensory tenderness (trained panel) and cooking losses among the experimental groups. Although they used another biological model, this study corroborates the results observed in the present study, in which dental carcass maturity did not influence carcass traits (Figure 1) or meat quality (Table 1).

The physiological age has been widely used by traditional meat-producing countries, considering the constant advances in finishing precocity of beef cattle. Studying the estimation of the age of cattle by the measurement of thermal stability of

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tendon collagen, Horgan (1991) concluded that, at slaughter, the animals appeared to be older than their real physiological age when evaluated by their dentition. This has been confirmed for a long time, such as when Wythes and Shorthose (1991) showed that, in cattle, the eighth teeth could erupt at any time between 39 and 57 months of age, depending only on the breed and nutritional management, factors that are determinant in the physiological age of individuals. Wiener and Purser (1957), Tulloh (1962) and Duarte et al. (2011) had already described that the better the nutrition conditions and selection process for physiological maturity, the earlier is the eruption of P.I. teeth.

We demonstrated that maturity, when evaluated by dentition (zero and two P.I. teeth), does not influence carcass traits and meat quality in non-castrated male F1 Angus-Nellore feedlot finished. Therefore, the carcasses of these animals should be subsidized in a similar way. Overall, Brazilian beef-packing companies could produce heavier and leaner carcasses of acceptable quality though the use of crossbred cattle such as non-castrated F1 Angus Nellore. Improvements in carcass weights could be made through the production of young bulls with two P.I., however, meat from bulls is commonly darker in color and less tender than meat from steers at heavier weights and advanced age. Alternatively, producer may use a greater feedlot finishing period (> 160 days) in order to partially compensate the deficiencies in young bulls' meat quality at heavier weights.

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Table 1. Meat quality traits of non-castrated male F1 Angus-Nellore cattle finished in feedlot with different numbers of permanent incisor teeth.

Variables (n = 88)[*]	Dental carcass maturity		SEM [†]	*P-value*
	0	2		
pH	5.75	5.72	0.03	0.557
L*	29.62	29.67	0.32	0.881
a*	15.73	16.08	0.24	0.544
b*	7.03	6.95	0.13	0.735
Chroma	17.31	17.52	0.27	0.819
Hue	23.64	23.40	0.15	0.419
Shear force, kg	5.43	5.44	0.11	0.969
Cooking loss, %	24.47	24.48	0.28	0.978
Moisture, %	73.00	73.18	0.11	0.434
Protein, %	22.58	22.51	0.06	0.542
Fat, %	3.31	3.21	0.11	0.664
Ash, %	1.10	1.09	0.00	0.190

[†] SEM, standard error of mean

[*] Both groups of animals were kept in the feedlot for 180 days. At the time of slaughter, 88 carcasses were selected, forming two treatments according to dental carcass maturity (0 versus 2 P.I. teeth; 44 animals per category).

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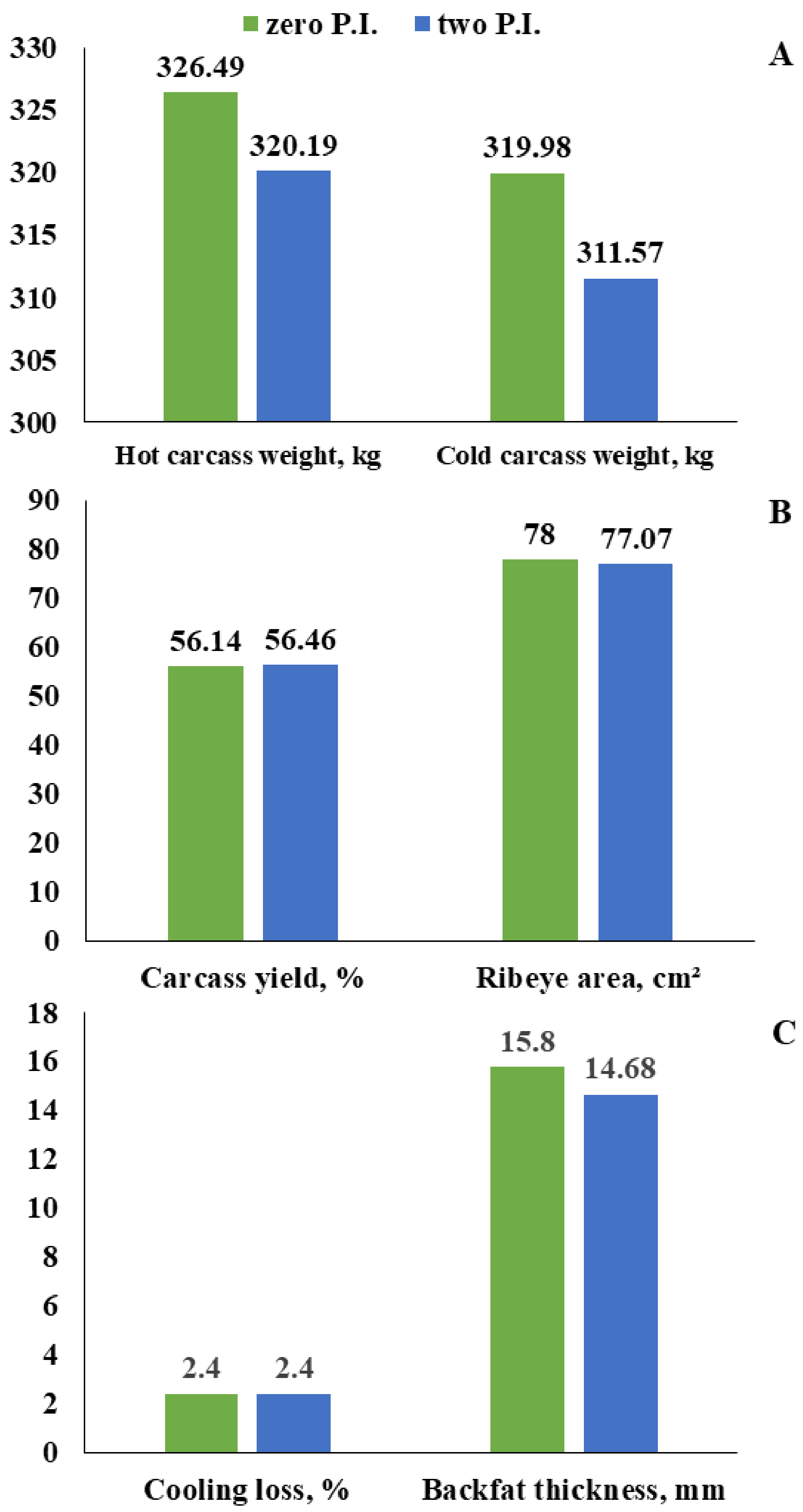

Figure 1. Carcass traits of non-castrated male F1 Angus-Nellore cattle finished in feedlot and slaughtered with zero versus permanent incisor (P.I.) teeth. Both groups of animals were kept in the feedlot for 180 days. At the time of slaughter, 88 carcasses were selected,

forming two treatments according to dental carcass maturity (0 versus 2 P.I. teeth; 44 animals per category). No difference ($P > 0.10$) was observed for carcass weights, carcass yield, carcass cooling loss, ribeye area and backfat thickness of the *Longissimus thoracis* muscle.

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Table S1. Experimental diet composition.

Ingredients	% Dry matter (DM)
Ground hay	13.98
Ground corn	68.76
Cotton seed cake	9.00
Peanut bran	2.05
Pre mixture (mineral and vitamin nucleus)	6.18
*Chemical composition**	
Dry matter (DM)	68.00
Crude protein (CP)	13.50
Ether extract (EE)	3.83
Neutral detergent fiber (NDF)	21.28
NEg [†]	1.30

[†] Net energy for gain (Mcal/kg DM).

* The composition of the diets was obtained by feed analysis (AOAC, 2005), followed by the procedures of determination of DM (method 976.05), CP (method 976.05, N * 6.25) and ash content (method 942.05). For NDF analysis, samples were treated with alpha amylase at a stable temperature without the addition of sodium sulfite and corrected for ash (Mertens et al., 2002). The EE analysis was conducted by the Soxhlet extraction (method 920.39).

ACCEPTED