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Effect of genotype and high roughage growing system on meat characteristics of Nellore and Zebu × Holstein crossbred cattle

Adailton Camêlo Costa
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Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Mario Luiz Chizzotti

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ABSTRACT

COSTA, Adailton Camêlo, D.Sc., Universidade Federal de Viçosa, June, 2025. **Effect of genotype and high roughage growing system on meat characteristics of Nellore and Zebu × Holstein crossbred cattle**. Adviser: Mario Luiz Chizzotti. Co-adviser: Cristina Mattos Veloso.

The objectives with this study were to: (1) evaluate the meat quality traits of crossbred animals (Zebu × Holstein) compared to purebred Nellore cattle slaughtered in a commercial abattoir; and (2) assess the effect of different growing-phase durations using high-forage diets on meat quality traits in early maturing Nellore steers. In the first study, the aim was to evaluate meat quality traits of purebred Nellore and F1 Zebu × Holstein crossbred cattle, considering the effect of postmortem aging from 0 to 14 days. Samples of the Longissimus thoracis muscle were collected from 20 Nellore and 20 Zebu × Holstein crossbred males, finished and slaughtered under commercial conditions. Samples were analyzed for instrumental color, thawing and cooking losses, Warner Bratzler shear force, myofibrillar fragmentation index (MFI), lipid oxidation, and proximate composition. Crossbred animals tended to present greater tenderness ($P = 0.051$) and lower thawing losses at the early postmortem stage. Aging for 14 days significantly ($P < 0.05$) reduced shear force and total exudative losses, while increasing ($P < 0.05$) the MFI in both genetic groups. Aged meat from crossbred animals achieved tenderness levels comparable to those of traditional beef breeds. Although aging modified color parameters and increased oxidative markers ($P < 0.05$), these remained within acceptable limits for sensory quality. No significant differences ($P > 0.05$) were observed in proximate composition between the genetic groups. It is concluded that Zebu × Holstein crossbreeding, combined with 14-day aging, significantly improves beef tenderness and water-holding capacity, representing an effective technological strategy to add value to male calves from dairy-origin herds. The second study investigated the effects of different durations of the growing phase (0, 28, 56, and 84 days), using high-forage diets, on meat quality traits of 36 early maturing Nellore young bulls with an initial body weight of 265 ± 5 kg. After the growing phase, all animals underwent an adaptation period and were finished on high-energy density diets, completing the production cycle, with slaughter performed at 154 days for all treatment groups. Average daily gain (ADG), carcass traits, and meat quality attributes were evaluated. Increasing the duration of the growing phase resulted in a linear decrease ($P < 0.001$) in ADG, hot and cold carcass weights, and carcass yield. However, the different growing durations did not significantly affect ($P > 0.05$) ultimate

pH, thawing and cooking losses, shear force, or proximate composition of the meat. An increase ($P < 0.05$) in lightness (L^*) of both meat and subcutaneous fat was observed with longer growing periods. The myofibrillar fragmentation index and lipid oxidation levels were influenced ($P < 0.05$) by the growing phase duration, although values remained within acceptable limits. It is concluded that, in short-cycle production systems, extending the growing phase with high-forage diets negatively impacts animal performance. Nevertheless, this strategy does not compromise most meat quality parameters, although it may affect proteolysis mechanisms and oxidative stability.

Keywords: *bos indicus*; breed; cattle; dairy herd; meat tenderness; postmortem aging; proteolysis

RESUMO

COSTA, Adailton Camêlo, D.Sc., Universidade Federal de Viçosa, junho de 2025. **Efeito do genótipo e do sistema de criação com alta forragem nas características da carne de bovinos Nelore e mestiços Zebu × Holandês**. Orientador: Mario Luiz Chizzotti. Coorientadora: Cristina Mattos Veloso.

Os objetivos com este estudo foram: (1) avaliar as características de qualidade da carne de animais cruzados (Zebu × Holandês) em comparação com bovinos puros da raça Nelore, abatidos em um frigorífico comercial; e (2) avaliar o efeito de diferentes durações da fase de recria utilizando dietas com alto teor de volumoso sobre a qualidade da carne de novilhos Nelore precoces. No primeiro estudo, o objetivo foi avaliar as características de qualidade da carne de bovinos puros Nelore e cruzados F1 Zebu × Holandês, considerando o efeito do envelhecimento pós-morte de 0 a 14 dias. Amostras do músculo Longissimus thoracis foram coletadas de 20 machos Nelore e 20 machos cruzados Zebu × Holandês, terminados e abatidos em condições comerciais. As amostras foram analisadas quanto à cor instrumental, perdas por descongelamento e cocção, força de cisalhamento, índice de fragmentação miofibrilar (IFM), oxidação lipídica e composição centesimal. Os animais cruzados tenderam a apresentar maior maciez ($P = 0,051$) e menores perdas por descongelamento no início do período pós-morte. O envelhecimento por 14 dias reduziu significativamente ($P < 0,05$) a força de cisalhamento e as perdas exsudativas totais, enquanto aumentou ($P < 0,05$) o IFM em ambos os grupos genéticos. A carne envelhecida dos animais cruzados alcançou níveis de maciez comparáveis aos das raças bovinas tradicionais. Embora o envelhecimento tenha modificado os parâmetros de cor e aumentado os marcadores de oxidação ($P < 0,05$), estes permaneceram dentro dos limites aceitáveis para a qualidade sensorial. Não foram observadas diferenças ($P > 0,05$) na composição centesimal entre os grupos genéticos. Conclui-se que o cruzamento Zebu × Holandês, combinado com o envelhecimento de 14 dias, melhora significativamente a maciez da carne e a capacidade de retenção de água, representando uma estratégia tecnológica eficaz para agregar valor aos bezerros machos de origem leiteira. No segundo estudo, foram investigados os efeitos de diferentes durações da fase de recria (0, 28, 56 e 84 dias), utilizando dietas com alto teor de volumoso, sobre as características de qualidade da carne de 36 novilhos Nelore precoces com peso corporal inicial de 265 ± 5 kg. Após a fase de recria, todos os animais passaram por um período de adaptação e foram terminados com dietas de alta densidade energética, completando o ciclo de produção, com abate realizado aos 154 dias para todos os

grupos. Foram avaliados ganho médio diário, características de carcaça e atributos de qualidade da carne. O aumento na duração da recria resultou em uma redução linear ($P < 0,001$) no ganho médio diário, nos pesos de carcaça quente e fria, e no rendimento de carcaça. Contudo, as diferentes durações da recria não afetaram significativamente ($P > 0,05$) o pH final, perdas por descongelamento e cocção, força de cisalhamento ou composição centesimal da carne. Observou-se aumento ($P < 0,05$) da luminosidade (L) tanto da carne quanto da gordura subcutânea com períodos de recria mais longos. O índice de fragmentação miofibrilar e os níveis de oxidação lipídica foram influenciados ($P < 0,05$) pelo período de recria, embora os valores tenham permanecido dentro dos limites aceitáveis. Conclui-se que, em sistemas de produção de ciclo curto, a extensão da fase de recria com dietas ricas em volumoso impacta negativamente o desempenho animal. Porém não compromete a maioria dos parâmetros de qualidade da carne, embora possa afetar mecanismos de proteólise e a estabilidade oxidativa.

Palavras-chave: *bos indicus*; bovinos; maciez da carne; maturação; proteólise; rebanho leiteiro; raça

SUMMARY

CHAPTER 1

General introduction

Brazil stands out as one of the leading producers and exporters of beef on the global stage, exporting significant volumes to key markets such as the United States and China. The country benefits from advantages such as extensive pasturelands and favorable climatic conditions that support the sustainable production of food. In this context, meat quality is essential to meet the export requirements of importing countries (Huang, L., 2025).

Beef quality can be influenced by various factors, including sensory attributes perceived by human senses such as sight, touch, taste, and smell. These are commonly associated with color, tenderness, juiciness, and flavor. Such attributes are affected by a combination of genetic, management, and technological factors. Genetic factors refer to inherent characteristics that cause differences among animals, such as breed, age, and sex, as well as physiological variations. Management factors involve aspects such as growth rate and nutritional strategies, particularly during the final finishing phase. Technological factors encompass all procedures and interventions applied to the animal from transport to slaughter, including storage, processing, and final consumption of the product (Clinquart et al., 2022; Ouali et al., 2006).

In Brazil, beef production systems are predominantly based on Zebu breeds, particularly Nellore cattle, due to their favorable meat production traits, adaptability, and resistance to parasites. Nellore cattle are commonly used in crossbreeding with dairy herds to produce heavier calves with improved carcass yield, thereby overcoming some of the limitations typically associated with meat from Zebu breeds (Abreu et al., 2017).

The practice of crossbreeding Nellore cattle with dairy breeds such as Holstein promotes increased heterosis and is widely used as a strategy to enhance meat quality in zebu animals and to improve carcass yield in dairy cattle. In the Brazilian context, this technique is extensively applied due to the improved precocity, faster growth rate, greater intramuscular fat deposition, and, consequently, enhanced meat tenderness compared to purebred zebu cattle. These factors contribute to improved sensory characteristics of the meat, better aligning with consumer market demands (Owens et al., 1995; Owens et al., 1993; Whipple et al., 1990; Koohmaraie, 1994; Martins et al., 2017).

Beef cattle finishing systems can generally be classified into pasture-based feedlot systems,

each with distinct characteristics that influence animal performance, meat quality, and production sustainability. Feedlot systems offer greater control over both diet and environmental conditions, enabling faster and more efficient weight gain, along with favorable meat quality outcomes (Patino et al., 2015). Additionally, this system allows for shorter finishing periods. In contrast, pasture-based systems are associated with higher sustainability and lower production costs and are widely adopted due to the abundance of natural pastures. However, they are subject to seasonal variations in the availability and nutritional quality of forage throughout the year (Silva et al., 2017). In this context, the use of preserved forages, such as sorghum silage during the growing phase in confinement, may represent a viable alternative strategy.

The sorghum genotype AGRI-002E is a Bolivian variety (AGRICOMSEEDS) known for its high dry matter yield and is widely used for silage production due to its low grain output (Da Rosa et al., 2022). However, studies evaluating its use during the growing phase of beef cattle and its impact on meat quality remain scarce.

Although the literature contains studies on the crossbreeding of Zebu cattle with dairy breeds, there is still a need for answers regarding the optimal genotype and production system to maximize the potential of crossbred animals for beef production. In addition, there is demand for growing and finishing systems with more favorable cost structures for meat production. Therefore, the objectives of this study were to: (1) evaluate the meat quality traits of crossbred animals (Zebu × Holstein) in comparison to purebred Nellore cattle, slaughtered in a commercial abattoir; and (2) assess the effect of different growing-phase durations using high-forage diets on meat quality in early maturing Nellore steers.

Keywords: *Bos indicus*, Breed, Cattle, Dairy herd

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CHAPTER 2

Effect of Nellore and Zebu x Holstein crossbreending on beef quality

Abstract

The valorization of male calves from dairy herds remains a challenge in beef production, with crossbreeding with beef breeds representing a promising alternative. This study aimed to evaluate the meat quality traits of purebred Nellore and F1 Zebu × Holstein crossbred cattle, considering the effect of postmortem aging from 0 to 14 days. *Longissimus thoracis* muscle samples were collected from 20 Nellore and 20 Zebu × Holstein crossbred males, finished and slaughtered under commercial conditions. Samples were analyzed for instrumental color, thawing and cooking losses, shear force, myofibrillar fragmentation index (MFI), lipid oxidation, and proximate composition. Crossbred animals tended to present greater tenderness ($P = 0.051$) and lower thawing losses at the early postmortem stage. Aging for 14 days significantly ($P < 0.05$) reduced shear force and total exudative losses, while increasing ($P < 0.05$) the MFI in both genetic groups. Aged meat from crossbred animals achieved tenderness levels comparable to those of traditional beef breeds. Although aging modified color parameters and increased oxidative markers ($P < 0.05$), these remained within acceptable limits for sensory quality. No differences ($P > 0.05$) were observed in proximate composition between the genetic groups. It is concluded that Zebu × Holstein crossbreeding, combined with 14-day aging, significantly improves beef tenderness and water-holding capacity, representing an effective technological strategy to add value to male calves from dairy origin herds.

Keywords: Bos indicus, Breed, Cattle, Dairy herd

1. Introduction

In most dairy production systems, the occurrence of male dairy calves being discarded and having no commercial value is common due to the high costs associated with keeping them in the production system until they attain commercial value. In several countries, these animals are slaughtered shortly after birth, which presents a problem for producers and the commercial dairy market, particularly considering pressures related to sustainable milk

production (Maher et al., 2021).

An alternative to improve the efficiency and utilization of male dairy animals for meat production is the use of crossbreeding dairy cows with beef bulls. This approach enhances economic returns, as there is significant demand for calves resulting from these crosses, as observed in the USA (Basiel and Félix, 2022).

In Brazil, the predominant breed for meat production is the Nellore, due to its good adaptability to tropical conditions, hardiness, resistance to parasites, and frequent use in studies and crossbreeding for quality meat production (Terto et al., 2012). The Holstein breed, a taurine breed, is widely distributed worldwide and serves as the basis for dairy production crossbreeding in the tropical regions (Abreu et al., 2017). The practice of crossbreeding between breeds or less closely related individuals ensures a greater degree of heterosis and is therefore used as a strategy to improve carcass yield and meat marbling.

In Brazil, this technique is primarily based on the precocity of the breeds, as British taurine animals, for example, generally exhibit faster growth rates, higher intramuscular fat deposition, and greater meat tenderness compared to Nellore animals (Owens et al., 1995; Owens et al., 1993; Whipple et al., 1990; Koohmaraie, 1994; Martins et al., 2017).

Due to the high number of cows in dairy production systems and the use of Nellore genotypes in crossbreeding for meat production, many crossbred animals are destined for slaughter.

The sensory quality of meat from crossbred animals of dairy origin represents one of the most promising aspects of this production strategy. According to Foraker et al. (2022), beef × dairy crossbred cattle exhibit intermediate characteristics between conventional beef cattle and purebred dairy animals, inheriting the musculature of the sire breed and the marbling typical of maternal dairy breeds. This combination results in meat products of superior quality, with most crossbred animals achieving a USDA Choice grade or higher. This represents an opportunity to increase the efficiency of production systems and improve carcass quality. However, further research is essential to evaluate different crossbreeding types and climatic conditions to produce high-quality dairy-origin beef.

Therefore, the objective with this study was to evaluate the meat quality characteristics of crossbred animals (Zebu x Holstein) compared to purebred Nellore animals finished and slaughtered in a commercial slaughterhouse.

2. Materials and Methods

In this study, samples of the *Longissimus thoracis* muscle were taken from 20 Nellore animals and 20 F1 Zebu x Holstein crossbred bulls. The animals were slaughtered at a commercial abattoir, with average cold carcass weights of 321.9 ± 26 kg and 320.8 ± 28 kg, respectively. After slaughter and carcass chilling, the carcasses were weighed, and pH was measured using a portable meat pH meter (Model HI 99163 – Hanna). Subsequently, two samples were taken from the *Longissimus thoracis* muscle between the 6th and 9th ribs of the left half of the carcasses. One sample from each carcass was frozen, while another was aged at 4º C for 14 days.

For the objective assessment of meat color, steaks with a thickness of 2.54 cm were thawed for 16 hours at 4°C. After this period, the samples were exposed to air and ambient temperature to allow for myoglobin oxygenation. A colorimeter (HunterLab MiniScan EZ 45/0 LAV) adjusted to the D65 light source was used to measure the L*, a*, and b* values according to the CIELab scale on the surface of the steak. Five readings were taken from each steak at different points, and an average was calculated for each sample. Wavelengths from 400 to 700 nm were captured in 10 nm intervals, and the reflectance ratio at 630 nm and 580 nm (R630/580) was used to assess color stability during display. Reflectance (R) at 473, 525, 572, and 700 nm was converted to absorbance (A) using the equation: A = log (1/R). The percentages of the three myoglobin redox forms—metmyoglobin (MMb), deoxymyoglobin (DMb), and oxymyoglobin (OMb)—were calculated following the AMSA (2012), equations.

$$\%MMb = \left(1{,}395 - \frac{A572 - A700}{A525 - A700}\right)$$

$$\%DMb = \left[2{,}35x\left(1 - \frac{A473 - A700}{A525 - A700}\right)\right]x100$$

$$\%OMb = 100 - (MMb + \%DMb)$$

The estimated values of Chroma and Hue were calculated using the equations provided in the American Meat Science Association's meat color measurement guidelines (King et al., 2022).

$$\text{Chroma} = [(a^*2 + b^*2)0.5]$$

$$\text{Hue} = [(\text{arctangent } (b^*/a^*)]$$

To evaluate exudative losses of the meat at time 0 and after 14 days of aging, steaks with a thickness of 2.54 cm were used. The steaks were weighed while frozen, then thawed and weighed again. The exudative losses were determined by the difference in weight. For cooking loss, the steaks were cooked in a water bath, set to 71°C for 40 minutes. After this period, the steaks were removed from the water bath and submerged in an ice bath for 1 minute to stop the cooking process. At the end of cooking, the steaks were weighed again. The losses were expressed as a percentage, using the following formula:

$$\text{Loss (\%)} = [(\text{weight before} - \text{weight after}) / \text{weight before}] \times 100$$

Shear force was measured on the same steak used for estimating losses. Five cylindrical samples, each 1.27 cm in diameter and parallel to the orientation of the muscle fibers, were extracted from each steak using a V-shaped cutting blade with a 60º angle, 1.016 mm thickness, and a fixed speed of 20 cm/min, attached to a TA.XT2i Texture Analyzer. The maximum forces required to shear the cylindrical samples were recorded, and the average of the five repetitions was used as the shear force value for each sample, serving as a parameter for tenderness.

The sarcomere length was estimated using the laser diffraction technique (Cross e West, 1981). Six muscle fibers were taken from each sample, placed on a glass slide, and a drop of 4°C sucrose solution was added to each fiber. A laser (632.8 nm) was directed onto the fibers, and six diffraction bands were recorded for each sample. The average of these values was used to calculate the sarcomere length according to the following equation:

$$\text{Sarcomere Length } (\mu\text{m}) = \frac{0{,}6328 x D x \sqrt{\left(\frac{T}{D}\right)^2 + 1}}{T}$$

Where: D = the distance in mm between the slide holder and the location where the laser diffraction bands are collected (150 mm), and T = the distance in mm between the extreme

bands divided by 2.

The myofibrillar fragmentation index (MFI) was determined according to the method described by Culler, Smith e Cross, (1978), with modifications by (Hopkins, Martin, Gilmour, 2004). Duplicate aliquots of 50 mg of muscle tissue, free of visible fat and connective tissue, were homogenized in 30 mL of ice-cold buffer (0.1 M KCl, 1 mM EGTA, 1 mM NaN_3, 1 mM $MgCl_2$, and 20 mM phosphate buffer at pH 7.0, 4 °C) using an Ultra-Turrax homogenizer at 19,000 rpm for two 30 s intervals, while keeping the samples on ice. The homogenate was filtered through a 1 mm² mesh screen and centrifuged at 1,000 × g for 10 min at 2 °C. This centrifugation step was repeated three times, with resuspension of the pellet in fresh buffer after each step. Protein concentration was adjusted to 0.5 mg/mL using the biuret method (Gornall, Bardawill, David, 1949). Absorbance was measured at 540 nm, and the MFI was calculated by multiplying the mean absorbance value by 150.

To determine the chemical composition of the meat, samples of the *Longissimus thoracis* muscle, approximately 90 grams each and free of subcutaneous fat, were minced, freeze-dried, and ground. These samples were then analyzed for moisture, crude protein, and mineral content using the methodology described by (Silva and Queiroz, 2002). The intramuscular fat content was analyzed in duplicate by extraction with petroleum ether, using Ankom XT4 filter bags and the Ankom XT15 fat extractor (ANKOM Technology, Macedon, NY, USA), following the manufacturer's recommendations.

For the evaluation of lipid oxidation, a homogenate (3 g/10 mL) of tissue was used to determine the concentrations of malondialdehyde (MDA) and nitric oxide (NO). The preparation was performed according to the methodology described by Walsh et al. (1993). Tissue lipid peroxidation was assessed by measuring MDA concentrations using the thiobarbituric acid reactive substances (TBARS) test, following the methodology described by (Buege and Aust, 1978). The results are expressed as nmol equivalents of MDA per gram of soluble protein.

The nitric oxide (NO) content was quantified indirectly using the standard Griess reaction (Tsikas, 2007). The results are expressed as µmol equivalents per gram of soluble protein.

The experimental design was completely randomized, with two genetic groups (Nellore and F1 Zebu × Holstein) and two postmortem aging periods (0 and 14 days), in a 2 × 2 factorial arrangement. Each group consisted of 20 animals.

Statistical analyses were performed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). For variables measured only once per animal, the model included the fixed

effect of breed:

$$Y_{ij}=\mu+R_i+\varepsilon_{ij}$$

Where Y_{ij} is the response variable, μ is the overall mean, R_i is the fixed effect of breed (I = 1, 2), and ε_{ij} is the random error.

For variables evaluated at both aging periods, the following model was used:

$$Y_{ijk}=\mu+R_i+T_j+(R\times T)_{ij}+\varepsilon_{ijk}$$

Where T_j is the fixed effect of again time (j= 0 or 14 days), and $(RxT)_{ij}$ is the interaction between breed and aging time. When significant differences were observed ($P < 0.05$), least square means (LSMeans) were compared using Tukey's test. Results are presented as LSMeans ± standard error, with statistical significance declared at $P < 0.05$.

3. Results and Discussion

Meat color is a highly relevant organoleptic characteristic in consumer purchasing decisions, as it is commonly associated with freshness perception (Tomasevic et al., 2021). It is a multifactorial attribute, influenced by both intrinsic factors—such as genetics and sex—and extrinsic factors, including diet, pre-slaughter handling, environmental conditions, and carcass chilling parameters (Malheiros et al., 2020).

According to Holman and Hopkins (2021), acceptable thresholds for beef color, based on data from various studies, include: L* > 31.4; a* > 14.5; b* > 6.3; hue angle > 22.5; and chroma > 17.4. In the present study (Table 1), the color values of the evaluated meat samples were consistent with consumer preferences. However, a significant effect of genetic group was observed on the L* value of the *Longissimus thoracis* muscle, with higher lightness recorded in samples from the Nellore group and a progressive increase in this parameter throughout the aging period. Similar findings were reported by Andrade et al. (2010), who described increases in L* values during postmortem aging in both Nellore and Red Norte cattle. During the aging process, the water-holding capacity (WHC) of the muscle significantly influences color parameters, particularly lightness. Changes in pH and alterations in the structure of myofibrillar proteins result in greater myofibrillar disorganization and increased extracellular water release (Redt, 2022). This release may explain the progressive increase in meat lightness over the aging period. Therefore, differences among breeds in

WHC can be attributed to adaptive physiological mechanisms. Breeds adapted to heat-stress environments, such as Nellore cattle, may exhibit physiological adjustments that enhance water retention and cellular homeostasis, thereby influencing meat lightness.

Regarding the a* and b* parameters, a significant reduction in red and yellow intensities was observed as aging progressed ($P<0.05$), along with a significant interaction between genetic group and aging time for the a* index ($P<0.05$), as shown in Table 1. These findings are consistent with those reported by Luciano et al. (2009), who observed a decline in a* values from 12 to below 8 after 14 days of aging, indicating a loss of color vividness. Beef color is primarily determined by the concentration and chemical state of myoglobin, a protein responsible for oxygen transport and storage in muscle tissues (Wicks et al., 2024). Several factors can affect meat color stability, one of which is lipid oxidation, which contributes to the deterioration of the red hue (Yim et al., 2016). According to Yar et al., (2024), in addition to the aging process, genotype and animal age significantly influence meat color traits. This may explain the observed interaction between aging and genetic group, which contributed to the reduction in the redness of the meat.

A significant effect of aging time on metmyoglobin content was observed, with a reduction in values at 14 days ($P = 0.0133$), as shown in Table 1. The significant decrease at 14 days in Nellore cattle may be associated with the activity of a higher concentration of reducing enzymes at the beginning of the aging period and low-oxygen conditions, representing a protective mechanism that helps maintain the visual quality of the meat during aging (King et al., 2011). Deoxymyoglobin levels also varied significantly ($P < 0.0001$), showing a marked increase over time. This result reflects fundamental adaptive processes that occur during postmortem aging. The accumulation of deoxymyoglobin in low partial oxygen pressure environments indicates reduced oxygenation of aged muscle and a potential decrease in color stability (Henriott et al., 2020). On the other hand, oxymyoglobin levels did not vary significantly ($P > 0.05$). Although no statistical effect was observed, higher concentrations of oxymyoglobin in meat are associated with a bright cherry-red appearance, which is preferred by consumers (Kiyimba et al, 2019).

The chroma index also showed a significant reduction with aging ($P < 0.05$), as presented in Table 1, indicating lower color saturation. A similar result was reported by Oliveira et al. (2021) in a study with Nellore × taurine crossbred cattle, where a decrease in chroma was observed up to 21 days of aging, confirming the loss of colorimetric saturation. This reduction during aging is related to oxidative processes occurring in muscle tissue. Prolonged oxygen

exposure and changes in muscle pH promote alterations in the chemical state of myoglobin, resulting in reduced intensity and purity of the red color characteristic of fresh meat (English et al., 2016). In the present study, the hue angle varied significantly between genetic groups ($P < 0.05$), with higher values observed in the crossbred group, indicating a greater tendency toward meat darkening. Variations in these indices are strongly associated with a* and b* values and are commonly used to estimate the degree of darkening or discoloration in muscle tissue (Mateus et al., 2018; Chen, 2023).

Carcass pH, evaluated 24 hours postmortem, remained within the physiological range considered ideal for beef (5.4–5.7), with no significant differences among treatments (Braden, 2013; Table 1). This pH stability indicates proper pre-slaughter handling and the absence of prolonged stress—both critical factors for ensuring final meat quality.

Regarding tenderness, Zebu × Holstein crossbred animals showed a trend toward greater meat tenderness ($P = 0.051$), along with a significant effect of aging time on this parameter ($P = 0.0001$), with the crossbred group exhibiting superior tenderness. This improvement may be attributed to increased postmortem enzymatic activity, particularly of the calpain–calpastatin system, as shown in Table 2. The literature reports that Zebu cattle, such as Nellore, exhibit high calpastatin activity, which inhibits the degradation of myofibrillar proteins and, consequently, compromises meat tenderness (Whipple et al., 1990; Koohmaraie, 1994; Martins et al., 2017). In contrast, taurine breeds and crossbreds tend to exhibit greater postmortem myofibrillar fragmentation, which contributes to improved texture and added value of the final product.

Shear force was significantly reduced with aging time ($P < 0.05$), as shown in Table 2, reaching values below 4 kgf classified as extremely tender meat according to Koohmaraie et al. (1994) and positively perceived by consumers (Liang et al., 2016). Aging thus stands out as an effective technological tool for standardizing meat tenderness, mitigating variations associated with production systems or genetic groups (Farias et al., 2018).

In Table 2, the myofibril fragmentation index (MFI) was also significantly influenced by aging, supporting studies that report increased proteolysis after 10 days of refrigerated storage (Fidelis et al., 2017). This degradation results from the activity of endogenous proteases, such as calpains and cathepsins, under chilling temperatures (0 to 1 °C) (Lage et al., 2009; Matheus et al., 2018). Aging has a significant impact on MFI, promoting greater fragmentation of myofibrillar structures over time. In a study by Ishihara et al., (2017), the use of meat cuts aged for different periods confirmed both improved tenderness and increased MFI values.

There was no significant difference in sarcomere length between genetic groups ($P > 0.05$), with average values of 1.46 μm observed. These values indicate sarcomere shortening induced by chilling, which helps explain the higher shear force values observed at day zero (Ertbjerg & Puolanne, 2017). This shortening results in greater resistance to cutting, negatively affecting the initial tenderness of the meat.

Thawing losses were lower in the Zebu × Holstein crossbred group at day zero ($P<0.05$), with a significant interaction between genetic group and aging time (Table 2). This lower loss observed in the crossbred animals may represent specific advantages in terms of meat quality, particularly regarding water-holding capacity (WHC), a trait that may be enhanced by crossbreeding Bos indicus with Bos taurus. Due to adaptive physiological processes, Nellore cattle tend to have a higher proportion of oxidative muscle fibers, which are associated with improved water retention. Reduced thawing and cooking losses offer important economic benefits for the meat industry, including higher processing yields, improved product appearance, and greater consumer satisfaction (Morón and Zamorano, 2003). Supporting this, studies conducted by Rubiano et al. (2009) reported lower thermal losses in meat from Nellore × Canchim crossbred animals.

Aging time significantly affected cooking losses ($P<0.05$), suggesting that aging enhances fluid retention during cooking due to the natural process of slight pH elevation and enzymatic degradation of the myofibrillar structure (Zeola et al., 2007). Total losses were not influenced by genetic group ($P>0.05$) but varied significantly according to aging time ($P<0.05$), with lower losses observed after 14 days of aging. In previous studies, Oliveira et al. (2021) reported that aging periods longer than 14 days increase drip losses, regardless of the type of Nellore × Taurine crossbreed. Such losses may be associated with myofibrillar degradation, ultimate pH, lipid content, and pre-slaughter stress response (Santos et al., 2013). Proteolytic degradation during aging leads to the release of soluble protein fragments, which contribute to exudate formation, thereby explaining the findings of the present study.

Lipid oxidation represents a critical factor in meat stability, negatively affecting the sensory, nutritional, and functional properties of the product. In the present study, there was a significant increase in malondialdehyde (MDA) levels with aging ($P<0.05$; Table 3). Despite this increase, the values remained below the sensorial threshold (0.5 mg/kg) and the rancidity threshold (1.5 to 2 mg/kg), as established by Spaziani et al. (2011) and Gray (1996).

Nitric oxide content (Table 3) also increased significantly with aging time ($P<0.05$), with no effect of genetic group. According to Hou (2019), nitric oxide synthase remains active up

to 14 days postmortem, catalyzing the conversion of L-arginine into citrulline and nitric oxide. This reactive molecule contributes to the formation of peroxynitrite, a highly oxidative compound that enhances lipid peroxidation (Rubbo, 1994). Literature also suggests that exogenous nitric oxide application may improve meat tenderness, whereas inhibition of nitric oxide reductase compromises this trait (Cook, 1998). In the present study, increased lipid oxidation, elevated nitric oxide levels, and improved tenderness were distinctive characteristics of aged meat.

Moisture, crude protein, intramuscular fat, and ash contents did not vary significantly among the genetic groups ($P>0.05$), as shown in Table 4. The moisture values were like those reported by Padre et al. (2006) for Nellore × Aberdeen Angus crossbreeds (~73.7%). The protein content of beef typically ranges between 21% and 24% (Prado et al., 2009), representing a strategic nutrient in the human diet. The lipid content observed in the Zebu × Holstein group were consistent with values reported for the Canchim breed (Prado et al., 2009). Ash values ranged from 3% to 4%, which aligns with the literature for various genetic groups finished in feedlot systems (Clímaco et al., 2011). Despite the adequate chemical composition, further studies are warranted regarding carcass yield, *Longissimus thoracis* muscle conformation, the suitability of production systems, and the development of effective incentive mechanisms for producers.

4. Conclusion

The crossbreeding between Zebu and Holstein cattle demonstrated positive effects on meat quality traits, particularly in terms of improved tenderness and reduced thawing losses during the initial postmortem period. Aging significantly enhanced tenderness, as evidenced by reduced shear force and increased myofibrillar fragmentation, rendering the meat from the crossbred group comparable to that of breeds traditionally used for beef production. Despite the observed decrease in color saturation and the increase in lipid oxidation and nitric oxide levels throughout the aging process, all values remained within acceptable sensory thresholds. The crossbred group also exhibited superior water-holding capacity, positioning it as a promising alternative for adding value to male calves from dairy herds. These findings underscore the technological potential of crossbreeding strategies combined with postmortem aging as effective approaches to improving the production of high-quality beef.

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Tables

Table 1. Color parameters of the *Longissimus thoracis* muscle from Nellore and Zebu x Holstein animals slaughtered in a commercial slaughterhouse.

	Zebu x Holstein		Nellore		*P- value*		
Item	0	14	0	14	Breed	Time	Breed*Time
Color *L**	32.37a±0.52	36.58b±0.52	34.39c±0.52	38.63d±0.52	0.0002	0.0001	0.9790
Color *a**	17.28a±0.45	14.83b±0.45	15.65b±0.45	15.07b±0.45	0.1335	0.0012	0.0425
Color *b**	23.54a±0.47	20.28b±0.47	22.50a±0.47	20.98b±0.47	0.1196	0.0001	0.2557
MMb	23.32a±3.94	21.49a±3.61	34.85b±3.61	18.85a±3.52	0.2425	0.0133	0.0578
DMb	16.18a±1.81	21.18b±1.66	13.95a±1.66	24.83b±1.62	0.7096	0.0001	0.0915
OMb	60.50±3.89	57.33±3.57	51.20±3.57	56.31±3.48	0.1596	0.7893	0.2582
Chroma	23.54a±0.47	20.28b±0.47	22.50a±0.47	20.98b±0.47	0.7201	0.0001	0.0684
Hue	47.20a±0.64	46.95a±0.64	43.87b±0.64	45.87a±0.64	0.0010	0.1758	0.0816
pH 24 hours	5.48±0.13	-	5.48±0.14	-	0.9826	-	-

L* - luminosity, a*- red intensity, b* yellow intensity, MMb – metmyoglobin, DMb – deoxymyoglobin, Omb – oxymyoglobin, [abc]LS means with uncommon superscript letter differ significantly ($P < 0.05$) by Tukey's test.

Table 2. Assessment of exudate losses, shear force, fragmentation index, and sarcomere length in Nellore and Zebu x Holstein animals slaughtered in a commercial slaughterhouse.

	Zebu x Holstein		Nellore			*P- value*		
Item	0	14	0	14	SEM[1]	Breed	Time	Breed*Time
Exudates losses, %	10.32a	12.32b	11.37b	11.60b	0.37	0.6470	0.0035	0.0192
Cooking losses, %	25.93a	22.36b	24.10c	22.18b	0.48	0.0343	0.0001	0.0909
Total losses, %	36.25a	34.68b	35.48a	33.78b	0.61	0.1607	0.0091	0.9143
Shear force, Kgf	6.53a	3.19b	7.13c	3.34db	0.19	0.0510	0.0001	0.2332
MFI[2]	19.42a	25.47b	16.09c	24.20b	1.13	0.0478	0.0001	0.3650
Sarcomere, µm	1.46	-	1.46	-	0.03	0.8982	-	-

[1]Standard error of the mean, [2]Myofibrillar fragmentation index, [abc]LS means with uncommon superscript letter differ significantly ($P < 0.05$) by Tukey's test.

Table 3. Assessment of lipid oxidation (Malondialdehyde - MDA) and nitric oxide (ON) quantity in the *Longissimus thoracis muscle* of Nellore and Zebu x Holstein animals slaughtered in a commercial slaughterhouse.

	Zebu x Holstein		Nellore		*P- value*		
Item	0	14	0	14	Breed	Time	Breed*Time
MDA, µmol/g	0.19a±0.009	0.31b±0.009	0.19a±0.009	0.33b±0.009	0.2858	0.0001	0.3876
NO, µM/g	0.85a±0.106	1.62b±0.106	0.90a±0.106	1.45b±0.106	0.5905	0.0001	0.2843

[abc]LS means with uncommon superscript letter differ significantly ($P < 0.05$) by Tukey's test.

Table 4. Chemical composition of the *Longissimus thoracis* muscle of Nellore and Zebu x Holstein cattle slaughtered in a commercial slaughterhouse

Item	Zebu x Holstein	Nellore	*P- value*
Moisture, %	73.45±0.2639	72.83±0.2572	0.0977
Protein, %	20.05±0.2943	20.87±0.2774	0.0627
Intramuscular fat, %	5.62±0.3948	4.84±0.4056	0.1778
Ash %	3.51±0.1299	3.56±0.1266	0.7637

CHAPTER 3

Effect of different growing periods using a high-roughage diet on meat quality of Nellore young bulls

Abstract

This study investigated the effects of different durations of the growing phase (0, 28, 56, and 84 days), using high-roughage diets, on meat quality traits of 36 early maturing Nellore young bulls with an initial body weight of 265 ± 5 kg. Following the growing phase, all animals underwent an adaptation period and were finished on high-energy density diets, completing the production cycle, with slaughter conducted at 154 days for all treatment groups. Average daily gain, carcass traits, and meat quality attributes were evaluated. Increasing the duration of the growing phase resulted in a linear decrease ($P<0.001$) in average daily gain, hot and cold carcass weights, and carcass yield. However, the different growing durations did not significantly affect ($P>0.005$) ultimate pH, thawing and cooking losses, shear force, or the proximate composition of the meat. An increase ($P<0.05$) in lightness (L) of both meat and subcutaneous fat was observed with longer growing periods. The myofibrillar fragmentation index and lipid oxidation levels were influenced ($P<0.05$) by the growing period, although values remained within acceptable limits. It is concluded that, in short-cycle production systems, extending the growing phase with high-roughage diets negatively impacts animal performance. Nevertheless, this strategy does not compromise most meat quality parameters, although it may affect proteolysis mechanisms and oxidative stability.

Keywords: Beef cattle, Meat color, Meat tenderness, Postmortem aging, Proteolysis, Meat color

1. Introduction

Beef cattle production represents one of the pillars of global meat supply, playing a strategic role in food security and national economic stability. Brazil has an estimated commercial herd of 186.9 million head, ranking second worldwide and establishing itself as the leading exporter of beef globally (USDA, 2025).

In recent years, short-cycle beef production systems have gained prominence, aiming to

reduce the time between birth and slaughter of cattle destined for meat production, thereby optimizing resource use and increasing productive efficiency (Valadares Filho et al., 2016). Although, in conventional systems, animals are still typically finished at around 24 months of age, it is technically feasible to shorten the production cycle to 12–14 months using energy dense diets. However, this strategy tends to be economically more demanding due to the high cost of feed inputs and the lower profit margins associated with intensive growing systems.

In this context, the use of diets with higher roughage content, particularly during the growing phase, has proven to be an effective strategy to promote compensatory growth in beef cattle, contributing to cost reduction at specific stages of the production cycle. This effect occurs following a period of undernutrition, when animals are subsequently re-fed, resulting in accelerated growth and a higher proportion of lean tissue (Kelly et al., 2016). In animals subjected to energy restriction, higher weight gain rates have been observed shortly after the beginning of the feedlot phase (Fontes et al., 2007; Almeida et al., 2001). This strategy can be implemented immediately after weaning, using feedlot systems as an alternative to reduce pasture occupancy by providing conserved roughage, such as sorghum silage, until the finishing period. Sustainable intensification practices have demonstrated effectiveness in reducing slaughter age without compromising animal performance (Millen & Arrigoni, 2013).

In addition to zootechnical efficiency, it is essential to assess the impact of these production systems on meat quality, considering that attributes such as pH, color, tenderness, and intramuscular fat content are directly influenced by nutritional and management factors, particularly during the final feeding stages (Koohmaraie & Geesink, 2006; Bressan et al., 2011; Aroeira et al., 2016).

Given this context, with the present study the aim was to evaluate the effect of different durations of the growing phase using high-roughage diets on meat quality traits of early-maturing Nellore young bulls.

2. Materials and Methods

This study was conducted at the experimental feedlot of the Department of Animal Science at the Federal University of Viçosa, in Viçosa, Minas Gerais, Brazil. All procedures were previously approved by the Institutional Animal Care and Use Committee of the Federal University of Viçosa (#032/2021).

Thirty-six early maturing Nellore young bulls with an initial body weight of 265 ± 5 kg

were used. The animals were randomly assigned to four treatment groups (n = 9 per group). Treatments consisted of different durations of the growing phase—0, 28, 56, and 84 days—using high-roughage diets, followed by a 14-day adaptation period to the finishing diet. Subsequently, animals underwent finishing periods of 140, 112, 84, and 56 days, respectively. Diets were formulated according to the recommendations of BR-CORTE (Valadares Filho et al., 2016) to achieve an average daily gain (ADG) of 0.6 kg/day during the growing phase and 1.2 kg/day during finishing (Table 1).

Animals were housed in covered collective pens (48 m² per pen), fed twice daily, and had ad libitum access to water. Body weight was recorded at the start of the experiment, after the growing phase, after the adaptation period, and again—following a 16-hour fasting period—prior to slaughter, to determine ADG and initial and final empty body weight. After 154 days of the experimental period, all animals were slaughtered following a 16-hour feed withdrawal, in accordance with Normative Instruction No. 9.013/MAPA (2017), ensuring compliance with sanitary regulations and animal welfare guidelines.

Figura 1. Experimental treatments

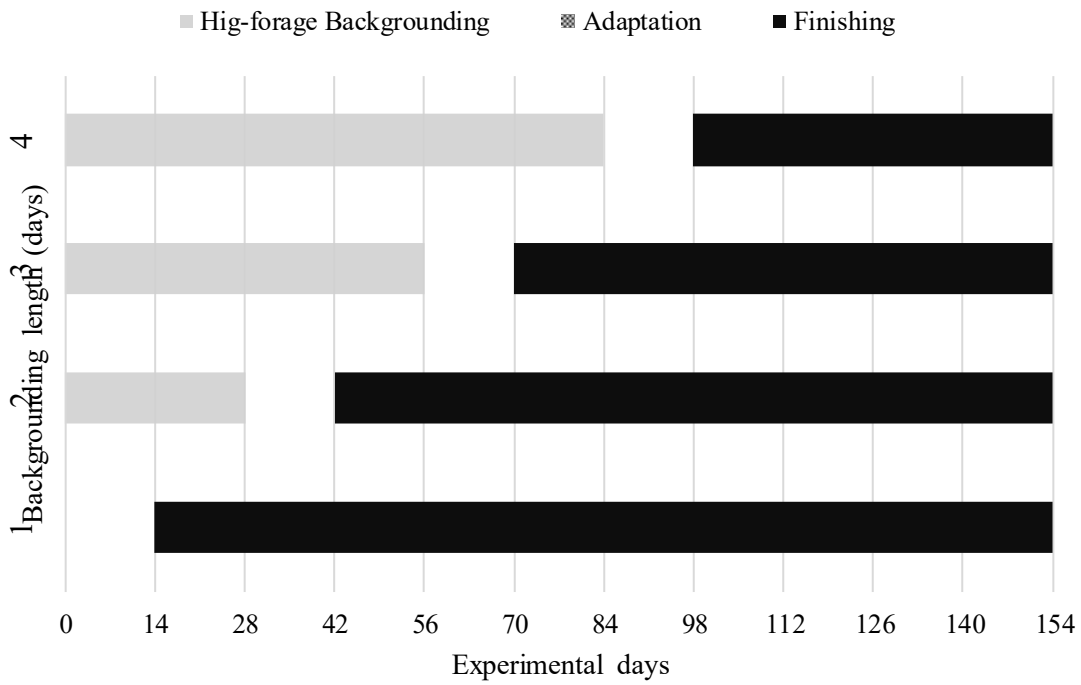

Table 1. Proportion of ingredients and nutrient composition of the experimental diets

Items	Diets	
	Backgrounding	Finishing
Ingredients, g/kg		
Corn Silage	-	200.0
Sorghum silage	800.0	-
Ground corn	136.6	740.4
Soybean meal	28.4	23.4
Urea	4.5	4.5
Ammonium sulfate	0.5	0.5
Virginiamycin	-	1.3
Mineral mix	30.0	30.0
Analyzed Composition, g/kg		
DM[1]	286.5	661.1
OM[3]	924.3	957.5
apNDF[4]	613.5	180.7
iNDF[5]	221.9	32.9
CP[6]	91.1	109.1
EE[7]	15.2	36.9
NFC[8]	212.6	638.8

[1]Mineral premix guarantees (per kg of DM): 190 - 240 g of Ca, 8.5 mg of Co (Min), 428 mg of Cu (Min),19 g of S (Min), 285 mg of Fe (Min), 10.3 g of P (Min), 21 mg of I (Min), 15 g of Mg (Min), 1285 mg of Mn (Min), 715 mg of monensin, 5.7 mg of Se (Min), 43 g of Na (Min), and 1714 mg of Zn (Min), 490g of protein nitrogen equivalent (Max). [2]Dry matter, [3]Organic matter, [4]Neutral detergent fiber corrected for ash and crude protein, [5]Indigestible neutral detergent fiber, [6]Crude protein, [7]Ether extract, [8]Non-fiber carbohydrates. In the "*Ingredients*" section, the unit g/kg represents grams of the ingredient per kilogram of dry matter in the diet. In the "*Analyzed composition*" section, the unit g/kg for dry matter represents grams of dry matter per kilogram of raw material, and for the other constituents represents grams of the constituent per kilogram of dry matter.

After slaughter, the carcasses were cleaned and split, and the left half-carcass was used to record hot carcass weight and measure muscle pH using a portable pH meter for meat (Model HI 99163 – Hanna). After chilling, the same measurements were repeated, and carcass yield

was calculated. Subcutaneous fat thickness was measured using a digital caliper. Subsequently, the Longissimus muscle was excised between the 6th and 9th ribs, from which three samples were collected. One sample (day 0) was immediately frozen, while the others were aged under refrigeration at 4°C for 7 and 14 days, respectively.

Meat Quality Analyses

For the objective evaluation of meat and fat color, steaks measuring 2.54 cm in thickness were thawed for 16 hours at 4°C. After thawing, samples were exposed to air at room temperature to allow for myoglobin oxygenation. Color measurements were performed using a colorimeter (HunterLab MiniScan EZ 45/0 LAV), calibrated to illuminant D65. The parameters L*, a*, and b* were recorded on the surface of each steak according to the CIELab color scale. Five readings were taken at different points on each steak, and the average value was used for statistical analysis. Spectral data were collected across the 400–700 nm range at 10 nm intervals. The reflectance ratio at 630 nm and 580 nm (R630/580) was used directly to assess color stability during display. Reflectance values (R) at 473, 525, 572, and 700 nm were converted to reflectance attenuation (A) using the equation: A = log(1/R). The relative proportions of the three redox forms of myoglobin—metmyoglobin (MMb), deoxymyoglobin (DMb), and oxymyoglobin (OMb)—were calculated according to the equations provided by AMSA (2012).

$$\%MMb = \left(1{,}395 - \frac{A572 - A700}{A525 - A700}\right)$$

$$\%DMb = \left[2{,}35x\left(1 - \frac{A473 - A700}{A525 - A700}\right)\right]x100$$

$$\%OMb = 100 - (MMb + \%DMb)$$

The estimated values of Chroma and Hue angle were calculated using the equations provided in the Meat Color Measurement Guidelines of the American Meat Science Association (King et al., 2022).

$$\text{Chroma} = [(a^*2 + b^*2)0.5]$$

$$\text{Hue} = [(\text{arctangent } (b^*/a^*)]$$

To evaluate drip loss at days 0, 7, and 14 of aging, steaks 2.54 cm thick were used. The steaks were initially weighed while frozen, then thawed and weighed again. Drip loss was calculated by the difference in weight before and after thawing. For cooking loss, steaks were cooked in a water bath set to 71°C for 40 minutes. After cooking, samples were immediately immersed in an ice bath for 1 minute to stop the cooking process. Steaks were then reweighed. Both drip and cooking losses were expressed as a percentage of the initial weight, according to the following formula:

$$\text{Loss (\%)} = [(\text{weight before} - \text{weight after}) / \text{weight before}] \times 100$$

Shear force was measured using the same steaks employed for loss estimations. Five cylindrical cores (1.27 cm in diameter) were obtained from each steak, cut parallel to the orientation of the muscle fibers. A V-shaped blade (angle: 60°; thickness: 1.016 mm) operating at a fixed speed of 20 cm/min was used, coupled to a TA.XT2i Texture Analyzer. The peak force (N) required to shear each core was recorded, and the average of the five measurements was used as the shear force value for the sample, representing the objective tenderness.

Sarcomere length was estimated using the laser diffraction technique described by Cross and West (1981). Six muscle fiber fragments were excised from each sample, placed on a glass slide, and a drop of cold sucrose solution (4°C) was added to each fiber. A helium-neon laser (λ = 632.8 nm) was directed at the fibers, and six diffraction patterns were recorded per sample. The average distance between diffraction bands was used to calculate the sarcomere length according to the following equation:

$$\text{Sarcomere Length } (\mu m) = \frac{0{,}6328 x D x \sqrt{\left(\frac{T}{D}\right)^2 + 1}}{T}$$

Where:

D = the distance, in millimeters, between the slide holder and the laser diffraction screen (150 mm); T = the distance, in millimeters, between the outermost diffraction bands divided by 2.

The myofibrillar fragmentation index (MFI) was determined according to the method described by Culler, Smith e Cross, (1978), with modifications by (Hopkins, Martin, Gilmour, 2004). Duplicate aliquots of 50 mg of muscle tissue, free of visible fat and connective tissue, were homogenized in 30 mL of ice-cold buffer (0.1 M KCl, 1 mM EGTA, 1 mM NaN_3, 1 mM $MgCl_2$, and 20 mM phosphate buffer at pH 7.0, 4 °C) using an Ultra-Turrax homogenizer at

19,000 rpm for two 30 s intervals, while keeping the samples on ice. The homogenate was filtered through a 1 mm² mesh screen and centrifuged at 1,000 × g for 10 min at 2 °C. This centrifugation step was repeated three times, with resuspension of the pellet in fresh buffer after each step. Protein concentration was adjusted to 0.5 mg/mL using the biuret method (Gornall, Bardawill, David, 1949). Absorbance was measured at 540 nm, and the MFI was calculated by multiplying the mean absorbance value by 150.

To determine the chemical composition of the meat, samples of the *Longissimus thoracis* muscle (approximately 90 g), free of subcutaneous fat, were finely chopped, freeze-dried, and ground. Moisture, crude protein, and ash contents were determined according to the procedures described by Silva and Queiroz (2002). The intramuscular fat content was determined in duplicate by petroleum ether extraction, using Ankom XT4 filter bags and the Ankom XT15 fat extractor (ANKOM Technology, Macedon, NY, USA), following the manufacturer's instructions.

Lipid oxidation was assessed using homogenized samples (3 g/10 mL of tissue) to determine the concentrations of malondialdehyde (MDA) and nitric oxide (NO). Sample preparation was conducted according to the method described by Walsh et al. (1993). Tissue lipid peroxidation was evaluated by measuring MDA concentrations using the thiobarbituric acid reactive substances (TBARS) assay, following the methodology of Buege and Aust (1978). The results were expressed as nmol of MDA equivalents per gram of soluble protein.

Nitric oxide (NO) levels were quantified indirectly through the Griess reaction, as described by Tsikas (2007), with results expressed as μmol of NO equivalents per gram of soluble protein.

The experimental design was a completely randomized design with four treatments (0, 28, 56, and 84 days of growing using a high-roughage diet) and nine replicates per treatment. For variables evaluated over aging time, a 4 × 3 factorial arrangement (four treatments × three aging times: 0, 7, and 14 days) was employed. Statistical analyses were performed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). For variables measured only once per animal, the following model was used:

$$Y_{ij}=\mu+T_i+\varepsilon_{ij}$$

where Y_{ij} is the response variable, μ is the overall mean, T_i is the fixed effect of treatment (i = 1 to 4), and εij is the random error, assumed to be normally distributed with homogeneous variance.

For variables measured at multiple aging times, the following model was applied:

$$Y_{ijk}=\mu+T_i+A_j+(T\times A)_{ij}+\varepsilon_{ijk}$$

where Aj is the fixed effect of aging time (j = 0, 7, and 14 days), and $(T\times A)_{ij}$ is the interaction between treatment and aging time. When significant effects were detected ($P < 0.05$), means were compared using Tukey's test. Results are presented as least square means (LSMeans), and statistical significance was declared at $P < 0.05$.

3. Results

Table 2 shows the effects of different durations of the growing phase using a high-forage diet (0, 28, 56, and 84 days) on performance, carcass yield, and meat quality parameters. Regarding average daily gain (ADG), animals fed the finishing diet immediately after the adaptation period exhibited the highest gain (1.12 kg/day). In contrast, those subjected to 28 and 56 days of growing with a high-forage diet had intermediate gains (0.90 kg/day), while animals in the 84-day growing group showed the lowest performance (0.60 kg/day), with a significant effect of the growing duration ($P < 0.001$). Empty body weight at the beginning (EBWi) and end (EBWf) of the trial did not differ significantly among treatments ($P = 0.999$ and $P = 0.053$, respectively). Hot and cold carcass weights were significantly lower in animals that underwent the longest growing period (84 days) ($P < 0.001$), presenting values of 216.77 kg and 214.21 kg, respectively. Initial and final pH values were not affected by treatments ($P > 0.05$). Carcass yield was significantly reduced in animals submitted to the longest growing period (59%), differing statistically from the other groups ($P < 0.020$). Subcutaneous fat thickness (SFT) and sarcomere length were not significantly affected by the duration of the growing period ($P > 0.05$).

Table 3 presents the effects of the growing period duration on meat quality traits, lipid oxidation, and nitric oxide content. Thawing loss, cooking loss, and total loss were not significantly affected by the duration of the growing phase ($P > 0.05$). However, all these variables were significantly influenced by aging time ($P = 0.001$, $P = 0.04$, and $P = 0.001$,

respectively), with no significant interaction between the factors. Even with aging up to 14 days, no significant differences were observed in meat tenderness ($P > 0.05$). The myofibrillar fragmentation index (MFI) was significantly affected by both growing period duration ($P = 0.0260$) and aging time ($P < 0.0001$), with a significant interaction between these factors ($P = 0.0040$). Lipid oxidation was influenced by the growing period ($P = 0.0028$) and aging time ($P < 0.0001$), with no significant interaction. Nitric oxide content was not affected by the duration of the growing period ($P = 0.1392$) but significantly increased with longer aging time ($P < 0.0001$).

Table 4 shows the results for meat and fat color parameters. Meat lightness (L*) was significantly influenced by both the number of days on the high forage growing diet ($P = 0.0004$) and the aging time ($P < 0.0001$). Animals subjected to 84 days of growing period showed higher L* values (40.03), indicating lighter meat compared to the other treatments (36.15–37.57). During the aging process, lightness increased up to seven days, with a significant difference observed ($P < 0.0001$). The meat color components a* (redness) and b* (yellowness) were not affected by the growing period duration ($P > 0.05$), although aging time had a significant effect on both parameters ($P < 0.0001$).

Color saturation (chroma) was not affected by the growing period ($P = 0.9169$), but decreased with aging ($P < 0.0001$), indicating a reduction in color vividness. Hue angle was influenced by both factors ($P < 0.05$). Regarding the redox forms of myoglobin, metmyoglobin (MMb) concentration increased significantly with aging time ($P = 0.0192$), particularly on day 14. In contrast, deoxymyoglobin (DMb) and oxymyoglobin (OMb) were not significantly affected by either the growing period or the aging time ($P > 0.05$).

As for fat color, the L* value increased significantly with longer growing periods ($P = 0.0348$), with the highest values observed in animals from the 84-day group. However, a decrease in fat lightness was observed during aging ($P = 0.0008$). The a* and b* values of fat were not affected by the growing period ($P > 0.05$), though the a* component increased significantly at day 14 of aging ($P < 0.0001$). Hue angle for fat was only influenced by aging time ($P < 0.0001$), with higher values observed at 14 days.

The results of the proximate composition are presented in Table 5. No significant differences were observed among treatments for any of the evaluated components—moisture, crude protein, ether extract, or ash regarding the duration of the growing period ($P > 0.05$).

4. Discussion

In this study (Table 2), increasing the duration of the high forage growing phase resulted in decreased average daily gain, final body weight, hot carcass weight, cold carcass weight, and carcass yield. These findings are consistent with results reported in the literature (Shibata, Hikino, & Matsumoto, 2018; Reuter & Beck, 2013; Ku et al., 2021). This outcome was expected due to the lower energy density of the high-forage diet during the growing phase, which limited animal performance despite being offered ad libitum. The group that was not subjected to the energy restriction period exhibited superior performance, reflected in greater final weight, heavier carcasses, and higher yield. This confirms that growing phases with higher dietary energy density are more advantageous for producing heavier and more developed carcasses (Han et al., 2024).

The inferior performance observed in the groups subjected to longer growing phases can be attributed to lower energy intake and insufficient time for compensatory gain during the finishing phase. This was a deliberate strategy to align the study with a short-cycle beef production system, which is why all animals were slaughtered after 154 days of experimentation (Table 2). Although the compensatory gain strategy is widely described in cattle subjected to moderate feed restriction (Galyean & Hales, 2023; Keogh et al., 2015), its effects are more pronounced when sufficient time and energy supply are provided during the realimentation period in the finishing phase. In the present study, the total feedlot period was relatively short for the groups with extended growing periods, which may have limited their ability to fully recover the reduced performance. Menezes and Fernandes (2019) reported that animals exposed to different growing strategies can yield comparable carcasses if finished to a common endpoint, which was not the case in this study.

The final pH values of the samples remained within the normal range (5.4 to 5.7), as shown in Table 2, and were similar across all treatment groups, indicating adequate nutritional status and absence of pre-slaughter stress (Braden, 2013). Sarcomere length was not significantly affected by the different growing periods. The sarcomere length values observed in this study suggest a potential occurrence of cold shortening. According to Ertbjerg and Puolanne (2017), values below 1.8 μm are considered indicative of cold-induced shortening. Although shear force values did not differ significantly across growing periods or aging times (Table 3), they may have been influenced by this possible cold shortening effect. Miller et al. (2001) reported that shear force values around 4.3 kg are associated with 86% consumer

acceptance, while values above 4.9 kg reduce acceptance to 25%. In this study, all animals produced meat with acceptable tenderness, which was not compromised by the different growing durations.

The results observed in Table 3 for thawing and cooking losses after a 7-day aging period can be attributed to the structural modifications that occur in the muscle matrix during meat aging. The action of proteolytic enzymes promotes the degradation of structural proteins, resulting in a reduction of water-holding capacity. These results demonstrate that total losses were greater at day 7 of aging and stabilized by day 14 (Pearce et al., 2011; Huff-Lonergan & Lonergan, 2005). When comparing cooking losses, aged meat exhibits higher losses than non-aged meat; approximately 5% of exudate is lost during aging periods of 3 to 8 days, according to Purslow et al. (2016), which may increase up to 9% in aging periods of 14 to 21 days. Losses during these processes are inevitable due to the presence of free water within the intercellular space of muscle tissues, which is released during thawing and cooking (Rahman et al., 2014).

Lipid oxidation (Table 3) increased with aging time, as described in the literature. The values obtained in this study are within the acceptable limits for meat oxidation, as reported by Spaziani, Stecchini, and Universita (2011) and Gray, Gomaa, and Buckley (1996), where rancidity indicators are above 1.5 mg/kg, and consumer-perceptible levels are around 0.5 mg/kg. These are positive indicators, suggesting that both the rearing duration and the aging time were not detrimental to meat quality.

The myofibrillar fragmentation index (MFI; Table 3) reflects the extent of myofibrillar degradation and is generally positively correlated with meat tenderness (Veiseth et al., 2001). At 0 and 7 days of aging, a noticeable increase in MFI was observed, a pattern explained by the activity of calpains, enzymes responsible for post-mortem myofibrillar degradation. However, the reduction of the index at day 14 represents a trend contrary to expectations, as continued fragmentation would be anticipated. This phenomenon may be explained by the increased nitric oxide levels observed at day 14, which may have inhibited calpain activity and the degradation of structural proteins such as desmin, thereby reducing myofibrillar fragmentation (Marino et al., 2015). The interaction of the fragmentation index suggests that rearing duration is not an isolated factor but interacts dynamically with the post-mortem aging process, modulating the degradation of the myofibrillar structure. In other animal models, feed restriction has been shown to influence the fragmentation index and muscle proteolysis by reducing the rate and extent of post-mortem myofibrillar fragmentation compared to animals fed ad libitum, possibly due to alterations in calpain system activity and calpastatin

levels (Leonardo et al., 2008).

Meat color is an important factor in consumer perception of quality. Beef from forage-fed cattle tends to exhibit fat with a yellowish or orange hue due to the presence of carotenoids in pastures or diets rich in roughage. This coloration is often negatively perceived at the point of sale, as consumers are accustomed to the bright red color typical of meat from animals fed grain-based diets (Duckett et al., 2013). According to Holman and Hopkins (2021), acceptance thresholds for beef color have been compiled from various studies, indicating that color is considered acceptable when L* > 31.4; a* > 14.5; b* > 6.3; Hue > 22.5; and Chroma > 17.4. In this study (Table 4), the color parameters were within acceptable levels, except for a*, which was slightly below the threshold. During the aging process, it is common to observe an increase in meat lightness, as seen in this study. Similar results were reported by Wicks et al. (2024), who observed increased lightness in meat from pasture-fed animals. This behavior may positively indicate that aging can contribute to the visual improvement of meat from cattle reared on high-roughage diets. In the present study, the values of a*, b*, chroma, and hue were reduced during aging. Mateus et al. (2018) found similar reductions in these parameters over the aging period. This phenomenon can be explained by biochemical changes in myoglobin, the primary pigment responsible for meat color. As aging progresses, myoglobin oxidation leads to the formation of metmyoglobin and the consequent reduction in meat color intensity (King et al., 2021). In research conducted by Wicks et al. (2024), animals finished under different pasture-based systems exhibited greater increases in lightness compared to those finished on grain-based systems, suggesting that high-roughage feeding systems can produce meat with visual characteristics similar to those obtained from high-grain feeding systems.

An increase in subcutaneous fat lightness was observed in this evaluation (Table 4) in the meat of animals subjected to longer rearing periods. This result may be related to the dilution of carotenoids deposited in tissues during the feeding phase with diets rich in roughage. Although the initial diet has the potential to intensify the yellowish coloration of fat, the subsequent lipid deposition during the finishing phase with concentrate may have reduced the concentration of these pigments, promoting a lighter appearance of the fat (Dunne et al., 2009).

The a* value of fat increased with aging time, possibly due to lipid oxidation reactions and interactions between residual pigments (Wood et al., 2008). Similarly, hue values increased over the aging period, which can be explained by oxidative processes occurring during

vacuum aging of fat, promoting color changes toward more yellowish and brownish tones (Mancini & Hunt, 2005).

Regarding the proximate composition of the meat, the results remained homogeneous among the experimental groups (Table 5). The ether extract content, indicative of intramuscular fat, was not higher even in animals that spent more time in the finishing period. Although diets with higher concentrate inclusion may favor greater marbling deposition, this was not observed in the present study (Petrick, Harper & Oddy, 2004).

5. Conclusion

In short production cycles of Nellore steers, extending the rearing phase with high-roughage diets for up to 56 days did not affect animal performance. However, longer rearing periods significantly reduced weight gain and carcass weight. Despite this reduction in performance, most meat quality traits were not compromised. An increase in myofibrillar degradation was also observed at 7 days of aging, indicating improved tenderness at this stage. Therefore, the adoption of high roughage rearing strategies should balance the reduction in productive performance with potential savings in feeding costs, given the limited time available for compensatory gains.

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Tables

Table 2. Performance and carcass traits of Nellore cattle subjected to different high-forage backgrounding durations.

Item	High-forage backgrounding duration				SEM[1]	*P- value*
	0	28	56	84		
Average daily gain, kg	1.12a	0.97b	0.91b	0.62c	0.53	<0.001
Initial body weight, kg	265.78	264.67	265.72	263.83	9.95	0.999
Final body weight, kg	442.56a	417.44a	408.5a	361.39b	15.00	0.053
Hot carcass weigth, kg	274.57a	257.64a	249.32a	216.77b	9.07	<0.001
Cold carcass weigth, kg	271.44a	254.67a	246.83a	214.21b	8.99	<0.001
Initial pH	6.68	6.67	6.71	6.75	0.07	0.875
Ultimate pH	5.76	5.82	5.92	5.78	0.10	0.670
Carcass yield, %	61.35a	61.00a	60.40a	59.37b	0.45	0.020
Backfat thickness, mm	4.44	3.10	3.79	2.47	0.68	0.216
Sarcomere, µm	1.28	1.29	1.19	1.32	0.07	0.631

[1] Standard error of the mean. [a,b] Means with different superscripts in the same row differ significantly ($P < 0.05$).

Table 3. Water losses, oxidative parameters, and myofibrillar fragmentation index of the *Longissimus thoracis* muscle from Nellore steers subjected to different durations of backgrounding with high-forage diets.

Item	High-forage backgrounding duration				SEM[1]	Aging time			SEM[1]	*P- value*		
	0	28	56	84		0	7	14		TMT	Time	TMT*Time
Exudates losses, %	6.750	6.836	6.200	7.766	0.402	6.6479a	7.9355b	6.08a	0.348	0.0559	0.0010	0.4503
Cooking losses, %	15.064	14.445	14.002	15.034	0.463	14.048a	15.454b	14.407ab	0.401	0.3094	0.0405	0.8684
Total losses, %	21.814	21.281	20.202	22.800	0.698	20.696a	23.389b	20.487a	0.604	0.0720	0.0013	0.8063
Shear force, Kgf	5.217	4.550	4.865	4.677	0.214	4.897	5.090	4.455	0.185	0.1440	0.0724	0.9812
MFI[2]	20.726A	22.035A	19.69B	22.187A	0.656	21.121a	23.035b	19.323c	0.568	0.0260	<0.0001	0.0040
MDA[3], µmol/g	0.161	0.169	0.152	0.156	0.091	0.136a	0.153b	0.183c	0.003	0.0828	<0.0001	0.1216
NO[4], µM/g	3.440	2.766	3.371	3.413	0.235	1.274a	3.216b	5.253c	0.203	0.1392	<0.0001	0.3558

[1]Standart error of the mean, [2]Myofibrillar fragmentation index, [3]Malondialdehyde, [4]Nitric oxide. [*a,b,c] Means with different superscripts in the same row differ significantly ($P < 0.05$). [A, B] Means with different uppercase superscripts in the same row differ significantly for backgrounding treatments ($P < 0.05$).

Table 4. Color parameters of the *Longissimus thoracis* muscle from Nellore steers subjected to different backgrounding with high-forage diets.

Item	High-forage backgrounding duration				SEM[1]	Aging time			SEM[1]	*P- value*		
	0	28	56	84		0	7	14		TMT	Time	TMT*Time
Meat color												
L*	36.157A	37.459A	37.578A	40.033B	0.6268	35.796a	38.412b	39.212ab	0.5428	0.0004	<0.0001	0.9908
a*	14.235	14.128	14.46	13.409	0.2924	15.452a	13.111b	13.61b	0.253	0.0713	<0.0001	0.8193
b*	13.102	13.409	13.22	13.879	0.2849	13.847a	12.554b	13.806a	0.2468	0.2362	0.0003	0.8173
Chroma	19.384	19.517	19.641	19.336	0.3338	20.777a	18.188b	19.443c	0.2891	0.9169	<0.0001	0.6496
Hue	47.441A	46.404A	47.494A	43.942B	0.7042	48.148a	46.27b	44.543c	0.6099	0.0014	0.0003	0.998
%MetMb	39.579	40.0891	34.415	38.723	2.0589	34.183a	39.217b	41.204b	1.783	0.1997	0.0192	0.8809
%DeoxyMb	13.667	13.565	13.951	13.853	0.6659	14.742	13.597	12.939	0.5766	0.9763	0.0872	0.651
%oOxyMb	46.457	46.345	51.535	47.349	1.9497	51.073	47.111	45.58	1.6885	0.1994	0.0646	0.8387
Fat color												
L*	68.294A	68.253A	68.851A	70.298B	0.5526	70.316a	68.78b	67.676b	0.4786	0.0348	0.0008	0.3723
a*	4.196	4.066	3.96	3.584	0.2112	3.422a	3.531a	4.902b	0.1829	0.2056	<0.0001	0.93
b*	15.409	14.552	14.898	15.071	0.2919	14.939	14.804	15.205	0.2527	0.2206	0.5242	0.9525
Chroma	16.203	15.45	15.467	15.3674	0.3286	15.32	15.493	16.052	0.284	0.5452	0.1699	0.9904
Hue	15.092	15.675	14.598	13.19	0.695	12.55a	13.377a	17.99b	0.6019	0.0788	<0.0001	0.5108

[1]Standard error of the mean. L* – lightness; a* – redness; b* – yellowness; MetMb – metmyoglobin; DeoxyMb – deoxymyoglobin; OxyMb – oxymyoglobin. [a,b,c] Means within a row with different lowercase superscript letters differ significantly ($P < 0.05$) for aging time. [A, B] Means within a row with different uppercase superscript letters differ significantly ($P < 0.05$) for backgrounding treatments.

Table 5. Proximate composition of the *Longissimus thoracis* muscle from Nellore steers subjected to different high-forage backgrounding durations.

Item	High-forage backgrounding duration				SEM[1]	*P-value*
	0	28	56	84		
Moisture, %	72.1662	72.1416	72.3318	72.594	0.5322	0.9199
Protein, %	21.1555	20.6033	20.2155	20.5633	0.3138	0.2247
Intramuscular fat, %	4.4366	4.8444	4.8677	4.5177	0.3822	0.7997
Ash, %	1.0377	1.0188	1.0288	1.0255	0.0173	0.8916