CRIS LUANA DE CASTRO NUNES

IMPACT OF CHILLING RATES ON BEEF QUALITY; THE POTENTIAL OF DEXA TO PREDICT FAT CONTENT; AND GENOMIC BREED TRACEABILITY OF CERTIFIED ANGUS BEEF SOLD IN BRAZIL

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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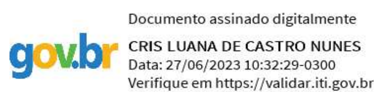

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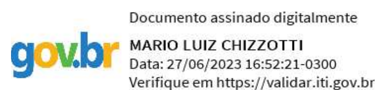

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“We cannot solve problems with the same thinking we used to create them”.

(Albert Einstein)

ABSTRACT

NUNES, Cris Luana de Castro, D.Sc., Universidade Federal de Viçosa, May, 2023. **Impact of chilling rates on beef quality; the potential of DEXA to predict fat content; and the genomic breed traceability of certified Angus beef sold in Brazil**. Adviser: Mario Luiz Chizzotti.

The objectives of this study were 1) to evaluate the temperature/pH decline response and meat quality parameters of *Bos indicus* beef carcasses subjected to conventional and dynamic chilling environments; 2) to assess dual-energy X-ray absorptiometry (DEXA) precision and accuracy in predicting *longissimus* steaks intramuscular fat (IMF) content; and 3) to determine the extent to which the Nellore breed contributes to the genetic composition of certified Angus beef and evaluate its relationship with the *longissimus* steaks composition from four different brands. In the first study, forty carcasses were used in each treatment (conventional and dynamic) to evaluate the effects of chilling decline rates on meat quality. Temperature and pH were recorded throughout the first 24h of chilling. After this period, *longissimus thoracis* was sampled, and meat quality analyses were performed. As results, carcass shrink percentage was higher in the conventional than in the dynamic chilling environment ($P = 0.049$). The pH values were significantly higher in the dynamic chilling environment at 2, 4, 6, and 12 hours after slaughter ($P < 0.05$). Also, there was a tendency ($P = 0.059$) for higher ultimate pH (24h) in the dynamic chilling environment. Temperature values were significantly lower in the dynamic treatment from 4 to 24 hours *postmortem* ($P < 0.001$) compared to the conventional treatment. Meat samples from the conventional chilling rate had higher mean values for color parameters a*, b*, oxymyoglobin, and chroma ($P \leq 0.006$). In contrast, the deoxymyoglobin value was higher in the dynamic chilling treatment ($P = 0.002$). The variation in chilling rate evaluated in this study impacted meat pH, color, and tenderness, as the dynamic chilling treatment resulted in a tendency towards higher ultimate pH levels, less intense red color with and higher shear force value. The second study was performed with 72 Angus and 72 Nellore steaks to assess the capability of DEXA to predict IMF content of beef *longissimus* against chemical IMF as the gold standard. DEXA performance of fat% prediction was assessed using a leave-one-out cross validation method among Angus and Nellore steaks, which generated a chemical fat% range of 14.05-36.82% and 2.46-7.84%, respectively, and using the pooled data. There was a significant positive association between DEXA predicted fat and chemical fat content. However, higher precision was found for pooled data ($R^2 = 0.95$, RMSECV = 1.95) and Angus (R^2

= 0.75, RMSECV = 2.39) than Nellore (R^2 = 0.15, RMSECV = 1.22) group. Accuracy also had the same response with average slope values close to 1 for pooled data and Angus and a lower value (0.42) for Nellore group. DEXA precisely predicts IMF content across a wide range of fat content. However, its precision and accuracy of prediction within low-fat content samples are lower than in high-fat content. Finally, to conduct the third study, DNA genotyping and chemical analysis were evaluated in 96 samples of Angus-certified meat, obtained from different brands, to determine the extent to which Nellore breed contributes to the genetic composition of certified Angus beef and evaluate the tissue composition of *longissimus* steaks from four different brands. On average, meat samples presented 53% of Angus genome and 47% of Nellore genome. There was a tendency for a negative correlation between Nellore proportion and fat content (P = 0.065), indicating that an increase in Nellore genetics may lead to lower fat content. Evaluating the chemical analysis among the four brands, Brand 2 presented the highest collagen (2,06%) and fat (7.97%) content values compared to Brand 3, which presented the least amount of collagen (1.69%) and fat content (5.14%). In addition, Brand 1 differed from Brand 2 on protein content (P = 0.047), in which Brand 1 presented a higher value (21.39%). On DEXA composition, Brand 3 presented the least fat content (12.58%) compared to the other brands. In sum, Nellore genetics contributed up to 49% of the genome profile of Angus-certified beef evaluated in this study. Also, there are differences in tissue composition between brands, with a tendency for leaner meat as the Nellore genetics increase.

Keywords: Beef carcass. Genotyping. Grading. Marbling. Meat quality. Tenderness. Temperature

RESUMO

NUNES, Cris Luana de Castro, D.Sc., Universidade Federal de Viçosa, maio de 2023. **Impacto das taxas de resfriamento na qualidade da carne bovina; potencial do DEXA para predizer o teor de gordura; e a rastreabilidade genética de raça em carnes Angus certificada comercializada no Brasil**. Orientador: Mario Luiz Chizzotti.

Os objetivos deste estudo foram: 1) avaliar as curvas de declínio de temperatura/pH e os parâmetros de qualidade da carne de carcaças de bovinos *Bos indicus* submetidas a ambientes de resfriamento lento e rápido; 2) avaliar a precisão e acurácia da absorciometria de raios-X de dupla energia (DEXA) na predição do teor de gordura intramuscular (IMF) de bifes l*ongissimus*; e 3) determinar até que ponto a raça Nelore contribui para a composição genética da carne Angus certificada e avaliar sua relação com a composição dos bifes *longissimus* de quatro marcas diferentes. No primeiro estudo, 40 carcaças em cada tratamento foram usadas para avaliar os efeitos da taxa de refrigeração lenta e rápida na qualidade da carne. A temperatura e o pH foram registrados durante as primeiras 24 horas de resfriamento. Após esse período, foi coletada uma amostra do *longissimus thoracis* e análises da qualidade da carne foram realizadas. Como resultados, a porcentagem de perda de peso das carcaças foi maior na taxa de resfriamento lento do que na taxa de resfriamento rápido (P = 0,049). Os valores de pH foram significativamente mais altos no ambiente de resfriamento rápido em 2, 4, 6 e 12 horas após o abate (P < 0,05). Além disso, obteve-se uma tendencia (P = 0,059) para pH final (24h) mais alto no resfriamento rápido do que no resfriamento lento. Os valores de temperatura foram significativamente menores no tratamento rápido de 4 a 24 horas após o abate (P < 0,001) em comparação com o tratamento lento. Nas amostras de carne, a taxa de resfriamento lento promoveu valores médios mais altos para os parâmetros de cor a*, b*, oximioglobina e croma ($P \leq$ 0,006). Em contrapartida, o valor de desoximioglobina foi maior no tratamento de resfriamento rápido do que no lento (P = 0,002). A variação na taxa de resfriamento avaliada neste estudo impactou o pH, a cor e maciez da carne, pois o tratamento de resfriamento dinâmico resultou em uma tendência a níveis de pH final mais altos, cor vermelha menos intensa e maior valor de força de cisalhamento. O segundo estudo foi realizado com 72 bifes Angus e 72 bifes Nelore para avaliar a capacidade da DEXA de predizer o conteúdo de IMF de bifes *longissimus* de carne bovina em comparação com o IMF químico como padrão ouro. O desempenho do DEXA na predição do percentual de gordura foi avaliado usando um método de validação cruzada *leave-one-out* entre os bifes Angus e Nelore, que gerou uma faixa de percentual

de gordura química de 14,05-36,82% e 2,46-7,84%, respectivamente, e usando dados agrupados. Houve uma associação significativa e positiva entre a gordura predita pelo DEXA e o teor de gordura química. No entanto, foi encontrada maior precisão para os dados agrupados (R^2 = 0,95, RMSECV = 1,95) e Angus (R^2 = 0,75, RMSECV = 2,39) do que para o grupo Nelore (R^2 = 0,15, RMSECV = 1,22). A acurácia também teve a mesma resposta com valores de *slope* próximos a 1 para dados agrupados e Angus e um valor mais baixo (0,42) para o grupo Nelore. O DEXA prediz com precisão o conteúdo de IMF em uma ampla faixa de conteúdo de gordura. Entretanto, sua precisão e acurácia na predição em amostras com baixo teor de gordura são menores do que em amostras com alto teor de gordura. Por fim, para realizar o terceiro estudo, foram realizadas genotipagem de DNA e análises químicas em 96 amostras de carne Angus certificada, obtidas de diferentes marcas, para determinar até que ponto a raça Nelore contribui para a composição genética da carne Angus certificada e avaliar diferenças em composição química entre as marcas. Em média, as amostras de carne apresentaram 53% do genoma Angus e 47% do genoma Nelore. Houve uma tendência de correlação negativa entre a proporção de Nelore e o teor de gordura (P = 0,065), indicando que um aumento na genética Nelore pode levar a um menor teor de gordura. Avaliando a análise química entre as quatro marcas, a Marca 2 apresentou os valores mais altos de colágeno (2,06%) e teor de gordura (7,97%) em comparação com a Marca 3, que apresentou a menor quantidade de colágeno (1,69%) e teor de gordura (5,14%). Além disso, a Marca 1 diferiu da Marca 2 no teor de proteína (P = 0,047), em que a Marca 1 apresentou um valor mais alto (21,39%). Na composição DEXA, a Marca 3 apresentou o menor teor de gordura (12,58%) em comparação com as demais. A genética Nelore contribuiu com até 47% do perfil genômico da carne bovina certificada Angus avaliada neste estudo. Além disso, há diferenças na composição do tecido entre as marcas, com uma tendência de carne mais magra com uma proporção maior de genética Nelore.

Palavras-chave: Carcaça bovina. Genotipagem. Tipificação. Marmoreio. Qualidade da Carne. Maciez. Temperatura.

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CHAPTER 1

General Introduction

Generally, meat consumers consider safety, price, and sensory quality as the main purchasing drivers (Hocquette, 2023; Liu et al., 2023). To meet consumer demands, the Brazilian beef industry has been searching for more efficient, economically viable and environmentally sustainable production models (Casagranda et al., 2023). Since Brazilian beef production is based on different production systems, such as intensive, extensive, and semi-intensive, coupled with an absence of a robust national carcass grading system, the meat industry faces challenges in achieving carcass homogeneity, particularly in terms of carcass fat content and size. Consequently, consumers often experience fluctuation in eating quality attributes.

Beef eating quality is affected by an association of multiple animal and environmental factors. Animal intrinsic factors such as genotype, gender, and age and environmental factors such as nutrition, production system (Rotta et al., 2009), pre-slaughter handling (England et al., 2013), and carcass processing (Zhang et al., 2019) can influence meat quality. In general, nutrition and sex affect mainly muscle area size and fat content. As a consequence of superior nutrition, for example, higher muscle mass and higher fat cover reduce temperature decline rate, mainly because fat cover provide a thermal insulation. Thus, temperature decline rate in larger and deep muscles is lower than superficially and flat muscles. Also, high muscle mass may elevate glycogen reserves, increasing the potential for pH decline (Ponnampalam et al., 2017).

The decline rate of temperature and pH in beef carcasses within the first 24h *postmortem* dictates the development of meat quality (White et al., 2006). Temperature and pH play an essential role in establishing *rigor mortis* and in the aging process by affecting mainly enzyme activity involved in the post-processes of biochemical reactions and proteolysis (Jerez-Timaure et al.,

2019). Therefore, knowing the decline pattern rate is important to predict meat quality parameters that the industry and consumers demand, such as meat color and tenderness.

The chilling protocol can influence the biochemical processes related to pH decline, as enzymes controlling the *rigor mortis* are temperature sensitive (Saleem & Majeed, 2014). Traditional chilling protocols, which involve cooling temperatures from 0 to 4ºC, are widely used in the meat industry. However, alternative chilling protocols have been studied to reduce the extent of cold shortening through slow chilling or to reduce carcass weight loss or chilling time through fast chilling (Zhang et al., 2019). As examples of slow protocols, carcasses are kept out of the cooler room for a certain period or elevated temperature conditions are applied, such as 10 to 16°C for 3 to 12h, or at a relatively lower, but still higher, than conventional chilling temperature (5 to 6°C) for 24h. On the other hand, fast chilling can be obtained when carcasses are kept in temperatures under -1ºC for a few hours post-mortem (Zhang et al., 2019). Nevertheless, carcasses subjected to these protocols may experience increased susceptibility to weight loss, meat toughening and darker color.

Moreover, another well-known factor contributing to beef eating quality is the variation between indicine and taurine breeds. Due to calpastatin activity, indicine cattle tend to produce a less tender beef than taurine after the same aging period (Ramos et al., 2020). Calpastatin is an endogenous calpain inhibitor. In the study of Wright et al. (2018), *Bos indicus* produced tougher meat than *Bos taurus* genotype based on shear force and panel scorer analysis due to higher activity of calpastatin, which decreases the degradation of myofibrillar proteins, including troponin-T and desmin, through calpain system. In addition, marbling scores on *Bos taurus* breeds tend to be greater than *Bos indicus* breeds (Pereira et al., 2015; Wright et al., 2018). However, the difference in marbling content between genotypes can also be due to diet, sex, days on feed, and age other than genotype.

Carcass grading is a fundamental process to determine the quality and value of animals at slaughter (Delgado-Pando et al., 2021). Grade assessment is the basis for transactions between producers and meat processors. In some countries, it also helps to determine the allocation of different cuts in the market, sorting into different niches. Thus, non-destructive, and objective methods for evaluating carcasses and meat offer enhancements to existing commercial subjective grading, which is susceptible to biases. The adoption of technologies based on X-ray (Calnan et al., 2021), nuclear magnetic resonance (Bernau et al., 2015), video image analysis (Hopkins et al., 2004), ultrasound (Aass et al., 2006), bioelectric impedance (Leighton et al., 2022; Zollinger et al., 2010), and spectroscopy (Kombolo-Ngah et al., 2023) have been evaluated for predicting marketable attributes such as yield, eating quality attributes, and carcass dimensions (Delgado-Pando et al., 2021).

In conclusion, there is a need for carcass and meat processing improvement due to market demands. Consumers' demand for high-quality food products is increasing, and their consumption decisions are becoming more complex. Simultaneously, the beef industry is also seeking more precise and objective ways to improve meat quality and grading. This allows for improved sorting and targeted marketing to specific niches, adding value and giving consumers a broader range of choices and enhanced eating experiences. Therefore, investigating current practices and exploring new technologies is essential to avoid profit loss and improve overall meat quality, sorting, and grading.

Thesis Objectives

The objectives of this thesis were to 1) evaluate the temperature/pH decline response and meat quality parameters of Bos indicus beef carcasses subjected to two chilling environments within the same abattoir, conventional and dynamic chilling environments; 2) assess DEXA precision and accuracy in predicting *longissimus* steaks IMF content across and within low- and high-fat content steaks using chemical fat analysis as the gold standard reference; and 3) determine the extent to which the Nellore breed contributes to the genetic composition of certified Angus beef and evaluate its relationship with the *longissimus* steaks composition from four different brands.

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CHAPTER 2

Impact of chilling decline rates on carcass and meat quality parameters of *Bos indicus* cattle

ABSTRACT

This study evaluated the impact of chilling decline rates on carcass and meat quality parameters of *Bos indicus* cattle. Eighty non-castrated Nellore half carcasses were used, divided equally into two treatments: conventional and dynamic chilling environment. The evaluations were performed over two days, where 20 carcasses from each treatment were evaluated daily. Temperature and pH were recorded at 0, 2, 4, 6, 12, and 24 hours in the *longissimus thoracis* muscle (LT) between the level of the 12th and 13th ribs. Cold carcass weight (CCW) was measured 24 hours after slaughter, and the LT of each left carcass was sampled. There was no effect of chilling environments on CCW. However, the shrink percentage was higher in the conventional than in the dynamic chilling environment ($P = 0.049$). The pH values were significantly higher in the dynamic chilling environment at 2, 4, 6, and 12 hours after slaughter ($P < 0.05$). Also, there was a tendency for high ultimate pH in the dynamic treatment ($P = 0.059$). Temperature values were significantly lower in the dynamic treatment from 4 to 24 hours *postmortem* ($P < 0.001$) compared to the conventional treatment. Carcasses subjected to the conventional chilling rate presented higher temperatures at pH 6 ($P < 0.001$), which was reached in a shorter period ($P = 0.024$). Carcasses in the conventional treatment had a lower pH at the temperature of 18ºC than in the dynamic chilling environment ($P < 0.001$). There were no differences in water losses and tenderness parameters between chilling environments ($P > 0.05$). However, meat samples from the conventional chilling environment had higher mean values for color parameters a*, b*, oxymyoglobin, and chroma ($P \leq 0.006$). In

contrast, the deoxymyoglobin value was higher in the dynamic than in the conventional chilling treatment ($P = 0.002$). The difference in chilling rates evaluated in this study did not affect the meat tenderness parameters of *Bos indicus* cattle. However, the variation in chilling rate impacted meat pH and color, with the dynamic chilling environment produce less bright red color.

Keywords: color, beef industry, Nellore, pH, temperature decline, tenderness

1. Introduction

During the first 24 hours *postmortem*, the chilling environment directly impacts carcass temperature and pH decline rates, which are considered critical regulators of the development of meat quality parameters (White et al., 2006). Temperature and pH play an important role in establishing *rigor mortis* and enzyme activity during the aging process, which can mainly affect meat color and tenderness (Jerez-Timaure et al., 2019).

According to the ideal temperature/pH window concept, beef carcasses should reach the pH value of 6 while the carcass temperature is still between 12°C and 35°C (Thompson, 2002). This concept emerged from the studies of Locker & Hagyard (1963), who demonstrated that myofibrillar shortening would increase when muscles were exposed to temperatures outside of this range. In agreement, Devine et al. (2002) showed that rigor at approximately 10-18ºC minimizes sarcomere shortening and maximizes aging potential, which leads to tender meat. Furthermore, some studies have shown that rapid chilling rates can result in darkening of the meat (Holdstock et al., 2023; Janz et al., 2002).

Traditional chilling protocols, temperatures ranging from 0 to 4ºC, are widely used in the meat industry. Different chilling protocols have been studied to reduce the extent of cold shortening or reduce carcass weight loss, e.g., delayed and fast chilling, respectively (Zhang et al., 2019).

However, the extent to which the chilling rate can be altered without causing noticeable darkening or toughening in beef is unknown (Holdstock et al., 2023). Because meat color and tenderness are key drivers of consumers purchasing decisions, chilling environments must be periodically evaluated to avoid low standards in meat quality.

Most studies evaluating the chilling effects on carcass and meat quality were conducted either on small ruminant carcasses (Hopkins et al., 2015; Devine et al., 2002; Vieira & Fernández, 2014) or *Bos taurus* carcasses (Holdstock et al., 2023; Haines et al., 2021; White et al., 2006), which are generally heavier and fattier than *Bos indicus* carcasses. Therefore, we hypothesized that slight increases in temperature decline rate on *Bos indicus* carcasses would yield tougher and darker meat. To test this hypothesis, our study aimed to evaluate the effect of conventional and dynamic chilling environments on the temperature/pH decline rate and meat quality parameters of *Bos indicus* carcasses.

2. Materials and Methods

Experimental design

This study was carried out in a commercial slaughter facility with export certificates. Chilling rates and meat quality parameters were evaluated on *Bos indicus* carcasses placed into two chilling environments: conventional and dynamic.

In the conventional environment, the chilling room was characterized by a steady setup of 4ºC for 24h and equipped with evaporators on one side. On the other hand, the dynamic chilling room had a programable temperature protocol starting with 9ºC, decreased by 1.425ºC per hour for the first 4h *postmortem*, then 3.3ºC until 24h. In this environment, the cooler room had evaporators on two opposite sides.

Eighty non-castrated *Bos indicus* Nellore carcasses were divided equally into the two treatment groups. Carcasses had an average age of 23.67±5.12 months old and an average cold carcass weight (CCW) of 296.2±16.7 kg. Fat cover was classified as scarce (1 to 3mm) and muscle conformation as regular, based on the commercial plant classification. The evaluations were performed over two days, where 20 carcasses from each treatment were evaluated daily.

Temperature, pH, and sample collection

Carcasses temperature and pH were recorded at 0, 2, 4, 6, 12, and 24h in the *longissimus thoracis* muscle (LT) between the level of the 12th and 13th ribs. The initial time (0h) was considered when the chilling room was filled and closed. A portable pH meter, Mettler Toledo Pro2Go, and a digital food thermometer (-50ºC to +300ºC) were used to measure pH and temperature, respectively. The pH meter was calibrated using pH standards of 4 and 7.

Following 48h of chilling, the LT of each left carcass was sampled, vacuum packaged individually, frozen, and stored at -20°C until subsequent meat quality analysis.

Meat quality analysis

The difference between the CCW and hot carcass weights (HCW) was used to calculate the 24h shrink percentage as follows:

$$Shrink\ percentage = (1 - (CCW - HCW)) \times 100$$

Meat quality analysis was performed at the Meat Science Laboratory of the Universidade Federal de Viçosa, MG, Brazil. One-inch steaks were obtained from the frozen LT portion using a butcher band saw. One steak was used for color measurements, while another was used for evaluating water losses, Warner-Bratzler shear force (WBSF), and sarcomere length.

For meat color measurement, steaks were thawed overnight at 4 °C, removed from vacuum packages, and exposed to oxygen 30 min prior to measurements. Values of L^* (lightness), a^* (redness), and b^* (yellowness) and the reflected light wavelengths were obtained from five readings performed at different points of the steak surface, using a Hunter MiniScan EZ colorimeter (4500L; Hunter Associates Laboratory, Inc., Reston, Virginia, USA) adjusted to the illuminant source D65 and observer 10º angle. The estimated chroma and hue values were calculated using the equations provided in the American Meat Science Association Meat Color Measurement Guidelines (King et al., 2022):

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

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

The wavelengths were used to determine the percentage of metmyoglobin (MMb); deoxymyoglobin (DMb), and oxymyoglobin (OMb), following AMSA equations (King et al., 2022):

$$\%MMb = \{1.395 - [(A572 - A730) \div (A525 - A730)]\} \times 100$$

$$\%DMb = \{2.375 \times [1 - (A474 - A730) \div (A525 - A730)]\} \times 100$$

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

The thawing loss was estimated by the weight difference between frozen and thawed steaks. Thawed steaks were vacuum-packed and cooked in a water bath at 70 °C for 30 minutes. Subsequently, the steaks were placed in an ice bath for 10 min to stop the cooking process and kept in the refrigerator for 24h. Lastly, steaks were removed from the package and weighed again to obtain water cooking loss. The total water loss in each steak was calculated using the following equation:

$$Total\ water\ loss\ (\%) = [(frozen\ steak\ weight - cooked\ steak\ weight)\ /\ frozen\ steak\ weight] \times 100.$$

To determine Warner-Bratzler shear force (WBSF), cooked steaks were cooled for 24h at 4°C, following the guidelines of the American Meat Science Association (AMSA, 1995). Six cylindrical samples, each 1.27 cm in diameter, were then removed from the steaks parallel to the long axis of the muscle fibers, using a stainless-steel device to extract samples (AMSA, 1995). The shear force was determined by perpendicular incision of the muscle fibers of each cylinder of meat by Warner-Bratzler shear device (G–R Electrical Manufacturing Company, Manhattan, KS, USA) equipped with a 1.1684 mm thick V-notched (60° angle) cutting blade at a constant speed of 2 mm/s and with a rechargeable 500 N digital force gauge (Mecmesin Basic Force Gauge) to measure and record peak force during sample processing.

Sarcomere length was estimated according to the laser diffraction technique (Cross et al., 1981). Six individual muscle fibers were teased from the muscle bundle and placed on a microscope slide with a drop of 0.2 M sucrose solution (0.2 M glucose and 0.1 M $NaHPO_4$ with pH 7). Sarcomere length was measured by laser diffraction using a 05-LHR-021 laser (Melles Griot, Carlsbad, CA) and calculated by using the following equation:

$$Sarcomere\ length\ (\mu m) = [0.6328 \times D \times \sqrt{(T/D)^2 + 1}]/T$$

In which: D = distance (mm) from the specimen-holding device to the screen (D had a constant value of 120 mm) and T = the separation (mm) between the zero and the first maximum band.

Statistical analysis

The data were analyzed on SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA), with each carcass treated as an experimental unit in a completely randomized design. Outliers were removed based on cold carcass weight using the SGPLOT procedure, eliminating

experimental units with CCW outside of the minimum and maximum fence of the Box Plot. After this step, 39 experimental units were retained for each treatment.

To estimate the decline rate in pH and temperature as a function of time after slaughter, we used the NLIN procedure according to the following exponential equation:

$$Y_{(t)} = A_{(i)} + (A_{(u)}*(exp^{(Kt)}))$$

In which $Y_{(t)}$ is the pH or temperature at time t; $A_{(i)}$ is the initial pH or temperature; $A_{(u)}$ is the ultimate pH or temperature the carcasses can reach; K is the decay rate; and t is the time in hours after slaughter.

The parameters $A_{(i)}$, $A_{(u)}$, and K were used to predict pH and temperature decline during the first 24 hours of carcass chilling, and to predict the pH in different temperature degrees (Figure 1 and 2, respectively), as well as the time in hours to reach pH 6 (time_pH6), the carcass temperature at pH 6 (temp@pH6), the time in hours to reach temperature 18ºC (time_temp18), and the carcass pH at temperature 18°C (pH@18). The relationships between temperature/pH parameters and beef quality measurements were performed using Pearson correlation (Table 1).

To evaluate the effects of chilling environments (conventional vs. dynamic) on carcass and meat quality parameters, we used mixed models (PROC MIXED) with initial carcass weight included as a covariate and slaughter day as a random effect to compare the mean of the pH and temperature parameters, following the statistical model below:

$$Y_{ijk} = \mu + T_i + cov\ (BCW) + D_{ij} + e_{ijk}$$

In which Yij is the response measured in carcass j submitted to treatment i; μ is the overall mean; T_i is the fixed effect of treatment (conventional or dynamic); $D_{(i)j}$ is the random effect associated with days of collection; and e_{ijk} is the residual error associated with each experimental unit.

The model used to evaluate CCW, HCW, shrink percentage, and meat quality parameters comprised the fixed effect of treatment and residual error. The least squares means (LSMEANS) were used to determine significant differences among treatments. Significance was declared at $P \leq 0.05$ and a tendency was reported if $0.05 < P < 0.10$.

3. Results

The estimated temperature and pH decline of conventional and dynamic chilling environments during the first 24h *postmortem* period and the pH decline alongside the temperature decline are shown in Figures 1 and 2, respectively. During the first 24h window, pH values of the dynamic chilling environment remained higher than the conventional chilling. Carcasses in the conventional environment reached pH6 at a higher temperature than carcasses in the dynamic environment (Figure 2).

Pearson correlation coefficients (Table 1) showed that significant correlations between temperature/pH (time_pH6, temp@pH6, time_temp18, and pH@temp18) and meat quality parameters varied between chilling environments. Percentage of MMb was negatively correlated with L*, a*, b*, and chroma ($r = -0.33, -0.47, -0.40$, and -0.50, respectively) values in the dynamic chilling environment. In contrast, there were no correlations between MMb and color parameters in the conventional chilling environment. Deoxymyoglobin was negatively correlated with meat color parameters (L*, a*, b*, and chroma), while OMb was positively correlated in both chilling environments.

Carcass and meat quality parameters are presented in Tables 2 and 3, respectively. There was a tendency ($P = 0.096$) for higher CCW in the conventional treatment. The shrink percentage was higher in conventional than dynamic chilling environments, 1.63 and 1.48%, respectively ($P = 0.049$), representing an average loss of 0.48 kg per carcass (Table 2).

There was a tendency ($P = 0.059$) for higher ultimate pH within the dynamic chilling environment. The pH values were significantly higher in the dynamic chilling environment at 2, 4, 6, and 12 hours after slaughter ($P < 0.05$), while temperature values were significantly lower in the dynamic treatment from 4 to 24 hours *postmortem* ($P < 0.001$) compared to the conventional treatment (Table 2).

Carcasses subjected to the conventional chilling presented higher temperatures at pH 6 ($P < 0.001$), which was reached in a shorter period ($P = 0.024$) than the dynamic environment, 2.51h and 5.75h, respectively. Furthermore, carcasses in the conventional treatment had a lower pH at the temperature of 18ºC than the dynamic treatment ($P < 0.001$), taking 0.87h longer to reach the same temperature ($P < 0.001$).

There were no differences in meat water losses between the chilling environments ($P > 0.05$) (Table 3). However, color parameters differed significantly between the two treatments. Meat samples from the conventional chilling environment had higher mean values for parameters a*, b*, oxymyoglobin, and Chroma ($P \leq 0.006$) (Table 3). In contrast, the deoxymyoglobin value was higher in the dynamic chilling treatment than in the conventional ($P = 0.002$) (Table 3).

There was no difference in sarcomere length between treatments ($P = 0.538$). However, there was a tendency ($P = 0.06$) for tougher meat in the dynamic chilling environment compared to the conventional chilling environment (Table 3).

4. Discussion

The decline in chilling rate significantly impacts meat pH, tenderness, and color, key quality parameters influencing purchasing decisions (Starkey et al., 2015; Mancini & Hunt, 2005). Factors such as the amount of cover fat, carcass size, spray chilling, and chilling protocol can affect the chilling rate (Djimsa et al., 2022; Park et al., 2007). However, it is unclear to what extent an

increase in chilling rate would negatively affect tenderness and color among carcasses with similar characteristics. In this study, we evaluate carcass and meat quality parameters in *Bos indicus* carcasses subjected to two different chilling rates, as can be seen in Figure 1 the treatments were effectively applied resulting in differences on temperature decay.

As expected, the dynamic chilling environment resulted in a lower shrink percentage. According to Savell et al. (2005), weight loss due to shrinkage can reach up to 2% of the hot carcass weight, causing major economic concern in the industry. The dynamic chilling rate in this study reduced 0.15% of the carcass shrinkage without a spray-chilling system, a well-known practice used to control carcass shrinkage. Other rapid chilling systems, which apply temperatures below freezing point, significantly reduce cooling time and shrinkage. However, these systems have also been associated with an increased proportion of darker and tougher meat, making them economically unfeasible (Aalhus et al., 2001; Savell. 2012; Zhang et al., 2019).

Under normal conditions, the pH falls from about 7 upon harvest to 5.4–5.7 during the first 24 hours *postmortem* in beef carcasses, with an increased temperature accelerating the rate of pH decline (Braden, 2013). Even though we found differences in pH values among treatments, the ultimate pH values were within the typical values range. In this study, there was, on average, 11ºC difference in the temp@pH6 between treatments and 3.24 hours difference to achieve the pH value of 6. Even so, both chilling environments achieved the goal established by the ideal temperature/pH window with apparently no impact on sarcomere length; however, with a tendency for higher shear force.

Also working with Nellore males, Malheiros et al. (2020) and Sant'Anna et al. (2019) found lower shear force compared to the values obtained in this study, 6.05 and 5.70 kg, respectively. Shear force values depend on the degree of myofibrillar contraction in the muscle after *rigor mortis*, exhibiting an inverse relationship (Lawrie & Ledward, 2014). According to the study of Destefanis

et al. (2008), the shear force values found in this study suggest that the evaluated beef may be considered tough. Based on consumer perceptions, Destefanis et al. (2008) study classified beef with WBSF values greater than 52.68 N (5.37 kg) as tough beef and according to Ertbjerg & Puolanne (2017) the sarcomere length of 1.4 found is this study is considered a shortened sarcomere.

Meat color is influenced by intrinsic factors such as genetics and sex and environmental factors, including diet, pre-slaughter management, and chilling conditions (Malheiros et al., 2020). Usually, consumers prefer bright cherry-red beef over darker cuts, which can be caused by ultimate pH above 6. The dynamic chilling environment examined in this study resulted in reduced redness, yellowness, and chroma of the meat and yet within the rage considered acceptable by the consumers. In the literature review performed by Holman & Hopkins (2021), consumer thresholds for beef color were compiled from different studies, which showed acceptable color when L* > 31.4; a* > 14.5; b* > 6.3; hue > 22.5; and chroma > 17.4. Even though consumers' color preferences can be affected by demographic factors (Zhang et al., 2021), the color parameters in this study are within those standard values. Understanding the acceptable standard for beef color is important for ensuring quality in the beef industry.

Holdstock et al. (2023) evaluated the effect of conventional (2ºC) and fast chilling, by removing the subcutaneous fat, on the color and quality characteristics of the *longissimus thoracis*. Their work showed the same pattern, at two days *postmortem*, found in this study for most of the parameters. Carcasses subjected to the faster rate of temperature decline (dynamic environment) had lower values of a*, b*, and chroma, lower proportion of oxymyoglobin, and increased deoxymyoglobin. However, they did not find differences in intramuscular pH decline rate between treatments on 0.75; 3; and 24 hours *postmortem*.

Less tender, darker in color, more purple-red and less saturated, and yet presenting a normal ultimate pH ($5.7 < pH \leq 6.0$) are also characteristics of atypical dark beef (Holdstock et al., 2014). This condition is not well characterized in the literature, and there is little investigation evaluating its effects on yield and eating quality (Mahmood et al., 2017). Comparing meat quality parameters between normal grade, atypical, and typical dark-cutting grade, Holdstock et al. (2014) observed that atypical dark-cutting grade carcasses had lower carcass weight than normal grade. Even though they did not evaluate the chilling environment, smaller or less muscled carcasses can be chilled more rapidly than heavier ones. So, the chilling rate can also be a potential cause for atypical dark-cutting despite a low glucidic potential.

Meat containing high levels of DMb is highly susceptible to oxidation. In the presence of oxygen, DMb reacts with oxygen radicals and reactive oxygen species, which causes rapid oxidation of DMb to MMb, even though the purple color of DMb may not be visible due to the excess of oxygen on the meat surface (King et al., 2022). Furthermore, lower temperature conditions during the early *postmortem* period preserve mitochondrial respiratory activity, which increases the proportion of DMb (Mancini & Ramanathan, 2014). As a result, meat with high DMb values exhibits less color stability and has a shorter shelf life than low-DMb meat due to the formation of a brown color, which is unappealing to consumers.

The rate of temperature decline in carcasses can be influenced by even slight changes in cooler temperatures, which primarily impact meat pH and color. The increase in beef carcass weight in recent decades has resulted in a broader range of hot carcass weights (Djimsa et al., 2022). Because of that, beef processing facilities have been seeking alternatives to achieve faster chilling rates and cool carcasses to the desirable deep tissue temperatures within 24 hours *postmortem*. However, accelerating the chilling rate for lighter carcasses with minimum fat cover can affect meat quality parameters (Djimsa et al., 2022). Our study provides insights into the effects

of chilling rate on meat quality, enabling us to inform industry practices to optimize meat processing for consistent quality outcomes.

5. Conclusion

The variation in chilling rate evaluated in this study impacted meat pH, color, and tenderness, as the dynamic chilling treatment resulted in a tendency towards higher ultimate pH levels, less intense red color with and higher shear force value.

Thus, establishing a chilling protocol based on the assessment of meat quality parameters is crucial to meet market demands, particularly considering that ultimate pH is a critical criterion for beef export to certain countries, and meat color is primary factor affecting consumer purchase decisions.

Conflict of interest

The authors declare no conflict of interest.

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Tables and Figures

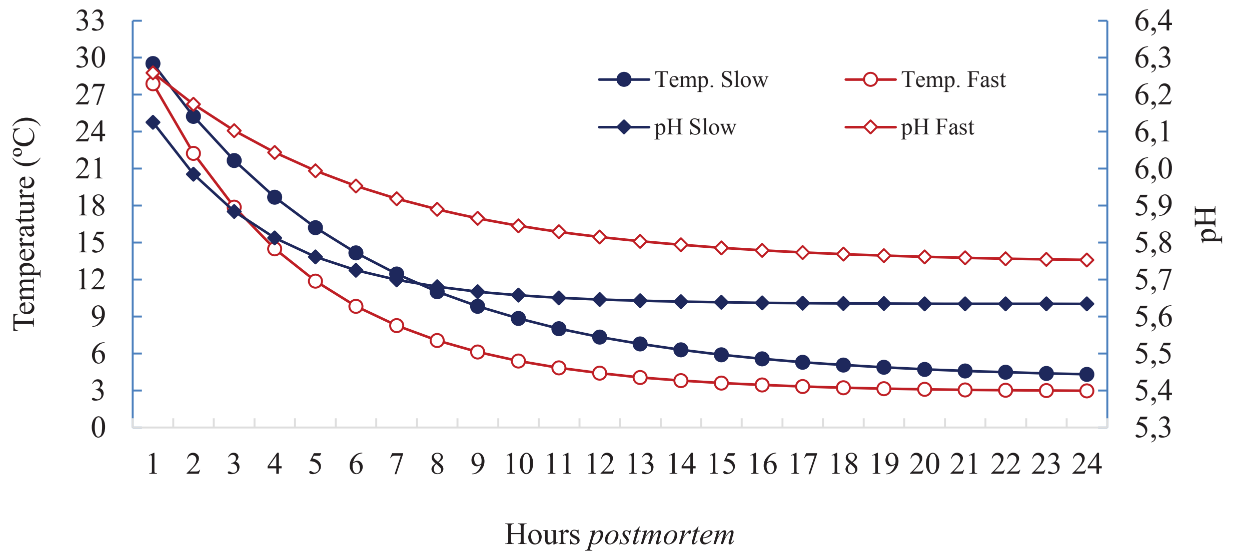

Figure 1. Estimated temperature and pH decline of conventional and dynamic chilling environments during the first 24h *postmortem* period

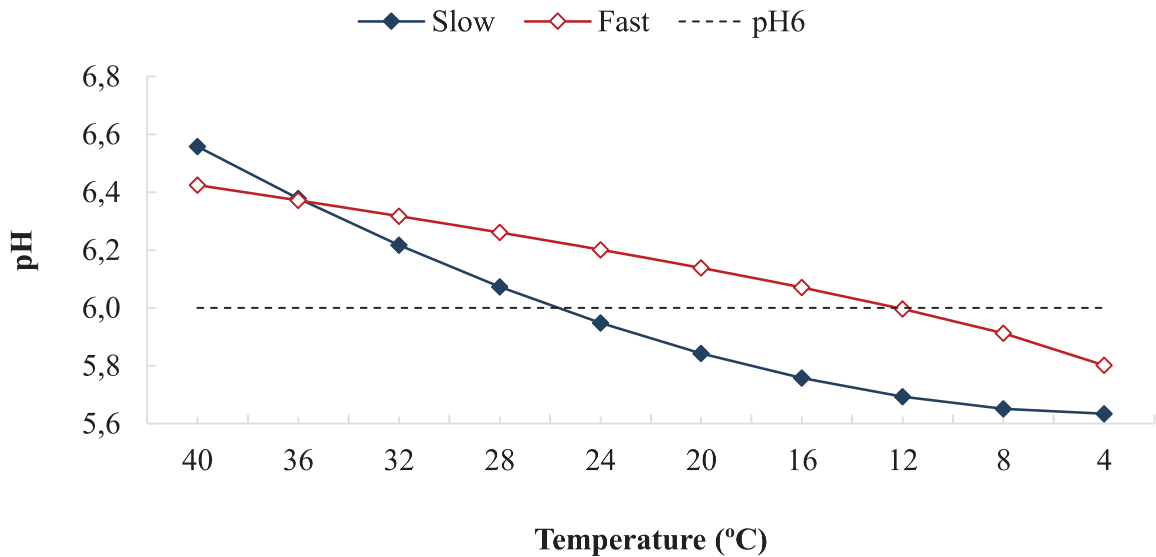

Figure 2. pH declines of conventional and dynamic chilling environments applied on the pH/temperature window.

Table 1. Pearson correlations and *P*-values indicating the relationships between temperature/pH parameters and beef quality measurements of dynamic (upper triangle in red) and conventional (bottom triangle in blue) chilling environments

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Time_pH6	1	**-0.567**	0.014	**0.623**	**-0.605**	0.096	-0.177	**-0.527**	-0.010	**-0.393**	-0.233	**0.418**	0.041	-0.182	0.125	**-0.491**	-0.316
		0.001	0.939	**<0.001**	**<0.001**	0.607	0.342	**0.002**	0.956	**0.029**	0.207	**0.019**	0.825	0.326	0.502	**0.005**	0.084
2. Temp@pH6	**-0.906**	1	0.259	**-0.950**	0.165	-0.162	-0.102	0.334	0.245	**0.424**	**0.392**	-0.239	-0.023	-0.056	0.055	-0.158	**0.413**
	<0.001		0.160	**<0.001**	0.375	0.383	0.585	0.067	0.185	**0.018**	**0.029**	0.195	0.902	0.764	0.770	0.395	**0.021**
3. Time_temp18	0.022	0.215	1	-0.247	0.033	**0.356**	**0.351**	-0.095	0.301	0.101	0.223	0.103	-0.112	-0.210	0.217	0.126	0.025
	0.897	0.208		0.180	0.849	**0.036**	**0.039**	0.593	0.084	0.571	0.205	0.562	0.527	0.234	0.217	0.472	0.886
4. pH@temp18	**0.823**	**-0.915**	-0.283	1	-0.183	0.196	0.130	**-0.369**	-0.225	**-0.460**	**-0.402**	0.291	-0.011	0.059	-0.041	0.108	**-0.449**
	<0.001	**<0.001**	0.094		0.324	0.291	0.486	**0.041**	0.223	**0.009**	**0.025**	0.112	0.952	0.753	0.825	0.563	**0.011**
5. TL	0.026	-0.168	**-0.689**	0.189	1	-0.187	**0.353**	**0.676**	-0.085	**0.596**	0.316	**-0.763**	-0.037	0.156	-0.103	**0.751**	0.106
	0.881	0.328	**<0.001**	0.269		0.254	**0.028**	**<0.001**	0.611	**<0.001**	0.053	**<0.001**	0.824	0.351	0.540	**<0.001**	0.522
6. CL	0.147	0.033	**0.797**	-0.132	**-0.585**	1	**0.853**	-0.264	0.125	**-0.424**	-0.199	**0.555**	0.081	-0.019	-0.023	0.146	**-0.361**
	0.391	0.848	**<0.001**	0.441	**<0.001**		**<0.001**	0.109	0.454	**0.008**	0.230	**<0.001**	0.629	0.909	0.891	0.375	**0.024**
7. TWL	0.191	-0.033	**0.649**	-0.079	-0.269	**0.938**	1	0.107	0.077	-0.084	-0.018	0.121	0.056	0.059	-0.071	**0.539**	-0.281
	0.265	0.849	**<0.001**	0.645	0.098	**<.0001**		0.522	0.647	0.618	0.915	0.469	0.738	0.727	0.670	**<0.001**	0.083
8. Color L*	0.298	-0.143	0.220	-0.007	-0.029	0.232	0.270	1	0.073	**0.769**	**0.511**	**-0.827**	**-0.337**	-0.046	0.192	**0.390**	**0.342**
	0.077	0.406	0.185	0.968	0.859	0.156	0.096		0.665	**<0.001**	**0.001**	**<0.001**	**0.039**	0.782	0.247	**0.016**	**0.036**
9. Color a*	-0.027	0.146	**0.633**	-0.294	**-0.538**	**0.551**	**0.425**	0.178	1	**0.502**	**0.846**	0.159	**-0.477**	**-0.667**	**0.736**	-0.138	**0.504**
	0.877	0.396	**<0.001**	0.082	**<0.001**	**<0.001**	**0.007**	0.279		**0.001**	**<0.001**	0.340	**0.002**	**<0.001**	**<0.001**	0.410	**0.001**
10. Color b*	-0.035	0.105	0.273	-0.308	-0.156	0.117	0.074	**0.455**	**0.739**	1	**0.885**	**-0.770**	**-0.403**	**-0.381**	**0.481**	0.248	**0.596**
	0.840	0.542	0.097	0.068	0.344	0.478	0.657	**0.004**	**<0.001**		**<0.001**	**<0.001**	**0.012**	**0.018**	**0.002**	0.133	**<0.001**
11. Chroma	-0.028	0.136	**0.527**	-0.315	**-0.417**	**0.407**	0.306	**0.319**	**0.958**	**0.900**	1	**-0.388**	**-0.506**	**-0.594**	**0.694**	0.072	**0.640**
	0.870	0.431	**0.001**	0.061	**0.008**	**0.010**	0.058	**0.048**	**<0.001**	**<0.001**		**0.016**	**0.001**	**<0.001**	**<0.001**	0.669	**<0.001**
12. Hue	-0.022	0.092	**0.588**	-0.036	**-0.598**	**0.640**	**0.504**	**-0.376**	**0.550**	-0.146	0.289	1	0.094	-0.043	-0.011	-0.431	-0.303
	0.900	0.592	**<0.001**	0.835	**<0.001**	**<0.001**	**0.001**	**0.018**	**<0.001**	0.375	0.075		0.574	0.798	0.949	0.007	0.064
13. MMb	**-0.507**	**0.417**	-0.176	**-0.386**	0.181	-0.249	-0.214	-0.214	-0.266	-0.224	-0.268	-0.117	1	0.265	**-0.669**	-0.007	-0.134

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	0.002	**0.012**	0.289	**0.020**	0.270	0.126	0.191	0.191	0.102	0.171	0.098	0.479		0.108	**<0.001**	0.967	0.421
14. DMb	0.023	-0.166	**-0.571**	0.309	**0.397**	**-0.421**	**-0.338**	**-0.378**	**-0.704**	**-0.580**	**-0.705**	-0.291	-0.067	1	**-0.894**	0.124	**-0.450**
	0.896	0.333	**<0.001**	0.066	**0.012**	**0.008**	**0.035**	**0.018**	**<0.001**	**<0.001**	**<0.001**	0.072	0.684		**<0.001**	0.457	**0.005**
15. OMb	0.267	-0.096	**0.594**	-0.041	**-0.447**	**0.505**	**0.412**	**0.448**	**0.763**	**0.631**	**0.765**	**0.319**	**-0.478**	**-0.844**	1	-0.093	**0.409**
	0.115	0.577	**<0.001**	0.811	**0.004**	**0.001**	**0.009**	**0.004**	**<0.001**	**<0.001**	**<0.001**	**0.048**	**0.002**	**<0.001**		0.580	**0.011**
16. WBSF	**0.387**	**-0.342**	0.058	**0.406**	0.205	0.217	0.346	0.005	-0.179	-0.276	-0.235	0.096	-0.328	0.206	-0.005	1	-0.107
	0.020	**0.041**	0.732	**0.014**	0.211	0.185	0.031	0.977	0.276	0.090	0.150	0.561	0.041	0.208	0.975		0.518
17. SL	**-0.394**	0.286	**-0.615**	-0.195	**0.457**	**-0.602**	**-0.507**	0.031	**-0.363**	-0.019	-0.242	**-0.515**	0.301	0.228	**-0.362**	**-0.433**	1
	0.019	0.096	**<0.001**	0.262	**0.004**	**<0.001**	**0.001**	0.854	**0.025**	0.908	0.143	**0.001**	0.066	0.169	**0.026**	**0.007**	

1. Time_pH6 (hours); 2. Temp@pH6 (ºC); 3. Time_temp18 (hours); 4. pH@temp18; 5. Thawing loss (%); 6. Cooking loss (%); 7. Total water loss (%); 8. Color L*; 9.Color a*; 10. Color b*; 11. Chroma; 12. Hue; 13. Metmyoglobin (%); 14. Deoxymyoglobin (%); 15. Oxymyoglobin (%); 16. Warner-Bratzler shear force (kgf); 17. Sarcomere length (µm).

Significant correlations ($P \leq 0.05$) are highlighted in bold.

Table 2. Effect of conventional and dynamic chilling rates on carcass, pH, and temperature parameters of *Bos indicus* beef carcasses.

	Chilling environments		SEM[1]	*P*-value
	Conventional	Dynamic		
Hot carcass weight (kg)	348.30	341.88	1.91	0.389
Cold carcass weight (kg)	342.16	336.22	1.89	0.096
Shrink percentage	1.63	1.48	0.04	0.049
pH				
0 h	6.35	6.38	0.02	0.519
2 h	5.99	6.15	0.03	0.014
4 h	5.84	6.08	0.03	0.001
6 h	5.81	6.01	0.03	<0.001
12 h	5.75	5.88	0.03	0.022
24 h	5.68	5.79	0.03	0.059
Temperature (ºC)				
0 h	34.78	34.99	0.32	0.753
2 h	24.19	23.24	0.30	0.139
4 h	18.07	15.27	0.31	<0.001
6 h	14.21	9.97	0.38	<0.001
12 h	7.66	4.83	0.28	<0.001
24 h	4.18	3.2	0.10	<0.001
Time_pH6 (hours)	2.51	5.75	0.82	<0.001
Temp@pH6 (ºC)	25.41	14.42	1.22	<0.001
Time_temp18 (hours)	4.15	3.28	0.12	<0.001
pH@temp18	5.81	6.07	0.03	<0.001

[1]SEM = Standard error of the mean.

Table 3. Effect of conventional and dynamic chilling rates on water losses, color, and tenderness parameters of *Bos indicus* beef carcasses.

	Chilling environments		SEM[1]	*P*-value
	Conventional	Dynamic		
Thawing loss (%)	7.91	8.26	0.28	0.533
Cooking loss (%)	22.1	21.79	0.52	0.763
Total water loss (%)	27.91	28.68	0.45	0.344
Color L*	36.41	35.74	0.30	0.270
Color a*	15.59	14.42	0.21	0.006
Color b*	13.55	12.41	0.19	0.002
Chroma	20.69	19.06	0.26	0.001
Hue	48.87	49.41	0.35	0.440
Metmyoglobin (%)	24.29	24.52	0.29	0.698
Deoxymyoglobin (%)	7.47	10.51	0.51	0.002
Oxymyoglobin (%)	68.24	64.97	0.60	0.006
WBSF (kgf)	7.11	7.94	0.22	0.060
Sarcomere length (μm)	1.42	1.43	0.01	0.538

[1]SEM = Standard error of the mean.

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CHAPTER 3

Assessing dual-energy X-ray absorptiometry prediction of intramuscular fat content in beef *longissimus* steaks

ABSTRACT

This study assessed the capability of dual-energy X-ray absorptiometry (DEXA) to predict intramuscular fat (IMF) content of beef *longissimus* steaks against chemical IMF as the gold standard. DEXA performance of fat% prediction was assessed using a leave-one-out cross validation method among Angus and Nellore steaks, which generated a chemical fat% range of 14.05-36.82% and 2.46-7.84%, respectively, and using pooled data. There was a significant positive association between DEXA predicted fat and chemical fat content. However, higher precision was found for pooled data (R^2 = 0.95, RMSECV = 1.95) and Angus (R^2 = 0.75, RMSECV = 2.39) than Nellore (R^2 = 0.15, RMSECV = 1.22) group. Accuracy also had the same response with average slope values close to 1 for pooled data and Angus and a lower value (0.42) for Nellore group. DEXA precisely predicts IMF content across a wide range of fat content. However, its precision and accuracy of prediction within low-fat content samples are lower than in high-fat content.

Keywords: composition; DEXA; grading; marbling; meat quality

1. Introduction

The development of non-invasive, fast, and reliable technologies to accurately evaluate carcass and meat quality parameters is one of the main objectives of the meat industry (Narsaiah et al., 2020; Delgado-Pando et al., 2021). Beef tenderness and palatability are strongly determined by the amount of IMF, also known as marbling, which can vary according to genetics, age, sex, and environment (Nguyen et al., 2021).

Marbling content plays an important role in meeting the market demand for meat quality standards (Cheng et al., 2015). Countries with an eating quality grade system evaluate marbling score as one of the carcass quality attributes, which is currently assessed subjectively through graders' scores or objectively with scanner technologies (Delgado-Pando et al., 2021). Subjective marbling score is assessed in an exposed rib eye section and performed using visual standard cards by accredited graders (Meat and Livestock Australia, 2018). However, visual grading scores can be considered inaccurate since they are subject to human error and bias (Hueth et al., 2007). Therefore, establishing an accurate technology to estimate IMF content as a quality attribute instead of the visual and single-site marbling score evaluation could improve cuts' sorting according to market demand.

Advanced technologies have been applied to objectively measure carcass characteristics that influence meat eating quality (Allen, 2021). Among them, DEXA technology provides information on bone, fat, and lean mass, and it is considered a contemporary method commonly used to assess human body composition (Fosbøl & Zerahn, 2015). Medical DEXA scanners have been used to evaluate the overall carcass or primal cuts composition of sheep (Pearce et al., 2009), beef (López-Campos et al., 2018), pigs (Soladoye et al., 2016), and chickens (Schallier et al., 2019). On top of that, DEXA's potential to predict lamb eating quality has also been assessed (Anderson et al., 2021). Most recently, DEXA technology has been adapted to overcome the medical DEXA

scanner practical limitation associated with speed and applied at chain speed in abattoir environments (Gardner et al., 2018; Calnan et al., 2021; Gardner et al., 2021). DEXA prediction of tissue composition can be used to substitute dissection and chemical analysis, which are laborious, sample destructive, time-consuming, and expensive to perform.

Most of the studies evaluating DEXA performance for fat prediction in beef and lamb carcasses and primal cuts have used dissected tissue or computed tomography scanning as the dependent variable (Gardner et al., 2018; López-Campos et al., 2018; Connaughton et al., 2020; Calnan et al., 2021). Therefore, we hypothesized that DEXA precisely and accurately predicts *longissimus* steaks IMF content across and within low- and high-fat content steaks using chemical fat analysis as the gold standard reference.

2. Material and Methods

Considering the IMF content variability between *Bos taurus* and *Bos indicus* cattle, 72 *Longissimus thoracis et lumborum* frozen steaks, one-inch thickness, from Angus crossbred cattle and 72 one-inch frozen steaks from Nellore cattle were selected to undergo DEXA composition and chemical fat content analysis. *Longissimus* steaks were obtained with the Certified Angus Beef brand from a commercial distributor. In contrast, Nellore steaks were obtained from animals finished in a feedlot trial at the Federal University of Viçosa, MG, Brazil. The steaks were obtained frozen and without cover fat but with the presence of the epimysium layer.

The frozen steaks were scanned in a medical DEXA unit (GE Healthcare, Lunar Prodigy Advance, USA), previously calibrated according to the manufacturer's recommendations, at the Body Composition and Densitometry laboratory of the Federal University of Viçosa. Before scanning procedures, the Small Animal configuration mode of the GE Healthcare enCORE software, version 18, was selected. Also, average sample weight and dimensions (width x length x

height) were inputted into the software before scanning. The DEXA device provided results for fat tissue mass (g), lean tissue mass (g), total tissue mass (g), and fat content (%).

Each steak sample was fully grounded using a meat grinder, weighed, and placed to freeze-dry (Liobras, model LP510, São Carlos, SP, Brazil). After water removal through the freeze-drying process, samples were weighed again and frozen in nitrogen liquid, then ground using a stainless ball mill (TECNAL, model R-TE-350, Piracicaba, SP, Brazil). Dry matter and fat content were determined using a subsample from the freeze-dried samples. The dry matter content was analyzed by drying the samples at 105 ± 2ºC overnight in an oven with forced air circulation (Detmann et al., 2012). The dry matter% was calculated by considering the two sequential drying procedures, freeze-drying and oven-drying, according to the following equations:

$$\text{Sample freeze-dried\%} = \frac{\text{final (dry) weight of the sample}}{\text{initial (wet) weight of the sample}} \text{ x } 100$$

$$\text{Sample oven-dried\%} = \frac{\text{final (dry) weight of the sample}}{\text{initial (wet) weight of the sample}} \text{ x}100$$

$$\text{Dry matter\%} = \frac{\text{Sample freeze-dried\% x Sample oven-dried\%}}{100}$$

Chemical fat analysis was performed in duplicate using Ankom XT4 filter bag and the fat extractor Ankom XT15 (ANKOM Technology, Macedon, NY, USA), which uses petroleum ether as solvent. Duplicates with a coefficient of variation above 10% in fat content were resampled from the complete ground sample and new duplicates were analyzed. Chemical fat content% was then calculated in wet tissue basis using the following equations:

$$\text{Fat content (g)} = \text{Sample weight before extraction (g)} - \text{Sample weight after extraction (g)}$$

$$\text{Fat content\%} = \frac{\text{Fat content (g)}}{\text{Sample weight before extraction (g)}} \text{ x } 100$$

$$\text{Fat content\% (dry basis)} = \frac{\text{Fat content\%}}{\text{Sample oven dried\%}} \text{ x } 100$$

$$Fat\ content\%\ (wet\ basis) = \frac{Fat\ content\%\ (dry\ basis)\ x\ Sample\ freeze\ dried\%}{100}$$

The SAS 9.4 software (SAS – Statistical Analysis Systems Institute Inc., Cary, NC, USA) was used to perform the statistical analyses. Analysis of variance (ANOVA) was performed to compare the dry matter and fat content between Angus crossbred and Nellore samples. Simple correlations were determined using PROC CORR between DEXA prediction and chemical analysis across all samples and within Angus and Nellore groups. General linear regression models were performed using the PROC REG procedure, according to the model:

$$Y = \beta 0 \pm \beta 1X + e$$

In which Y is the observed fat represented by the chemical analysis; $\beta 0$ and $\beta 1$ are the regression components; X is the predicted fat content by DEXA scanning; and e represents the random error. Data are presented as mean ± standard error of the mean (SEM) and significant differences were accepted if $P \leq 0.05$. DEXA fat content was used to predict chemical fat analysis across all samples and within breeds. The precision of the predictions was assessed based on the coefficient of determination (R^2) and root mean square error (RMSE).

To ensure the robustness of the models, a leave-one-out 5-fold cross-validation was performed using the PROC GLMSELECT procedure in SAS. In this analysis, each group (pooled samples, Angus and Nellore) was divided into 5 equal parts. One of the parts was held out for validation, and the model was fitted on the remaining 4 parts. This process was repeated 5 times until models had been validated in each of the 5 groups separately, generating a total of 5 validation tests. For the relationship between actual versus predicted, bias was presented as the difference between actual minus predicted values at the mean of fat% observations. Bias and slope are shown as indicators of accuracy while R^2 and root mean square error of cross validation (RMSECV) are shown as indicators of precision.

3. Results and Discussion

The simple correlation between DEXA values and chemical analyses across all samples is shown in table 1. There was a high and significant correlation between DEXA fat prediction and chemical fat content as % of wet sample ($r = 0.979$; $P < 0.001$). However, when the correlation was evaluated within Angus and Nellore groups, the strength of the correlations differed (Tables 2 and 3). Angus crossbred steaks had a stronger correlation ($r = 0.868$; $P < 0.001$) than Nellore steaks ($r = 0.390$; $P < 0.001$).

Across all samples, a wide range of fat content was generated, ranging between 2.46 and 36.82% on a wet basis (Table 4). Also, genotype reflected differences in fat content, Angus crossbred steaks presented higher fat content than Nellore steaks, generating two divergent groups. Even though *Bos taurus* breeds tend to have superior weight gain, greater fat thickness, marbling scores, and tender meat over *Bos indicus* breeds (Pereira et al., 2015), the difference in IMF% could be related to factors other than breed such as diet, sex, days on feed, and age, for instance.

In this study, comparing the percentage of fat content on a wet basis between observed and predicted, DEXA overestimated chemical fat analysis by 2.74 and 2.62% in Angus crossbred and Nellore samples, respectively (Table 4). On the other hand, studying the prediction of the 9th to 11th rib section composition by DEXA scanning, Prados et al. (2016) found DEXA underestimation of chemical fat content by 212g and overestimation of free fat tissue by 258.7g, which represented 6.12 and 7.46%, respectively, in relation to total tissue. The fat content underestimation could be due to imprecision in the dissection method used to separate meat and bone tissues from the rib sections prior to chemical analysis. The authors also demonstrated that equations fitted for a specific breed (e.g., Angus) might not fit for another breed (e.g., Nellore and crossbred Nellore x Angus) because of variation in tissue composition. Lukaski et al. (1999)

associated the DEXA underestimation of fat mass in pigs' body composition found in their study with the bone presence, which could function as a barrier for DEXA fat tissue prediction.

Although the model's prediction of chemical IMF content fitted across all samples described 96% of the variation with an RMSE value of 1.95 (Figure 1), there was a variation in performance when the model was fitted within each genotype group (Figure 2). The regression equation to predict fat content on Angus crossbred samples presented a better precision of prediction (R^2 = 0.75; RMSE = 2.39) than the equation on Nellore samples (R^2 = 0.15; RMSE = 1.22). However, Soladoye et al. (2016) presented equations with $R^2 > 0.80$ in their study evaluating DEXA prediction of total dissected fat of pigs carcasses from different sire breeds with different fat contents. In a literature review performed by Scholz et al. (2015), DEXA prediction accuracy was compared between different studies. They observed that samples with a low amount of fat mass and tissue dissection as the reference method led to a larger prediction error.

Kipper et al. (2019) also used a medical DEXA device to predict pig carcass and primal cuts composition. Predicting dissected fat mass of primal cuts, the authors found R^2 values ranging from 0.89 to 0.97 across all primal cuts. Therefore, it shows that DEXA has high precision in assessing dissected fat composition. In another study, Ribeiro et al. (2011) evaluated DEXA precision of prediction of dissected (R^2 = 0.84; RMSE = 2.94) and chemical (R^2 = 0.86; RMSE = 3.25) fat of rib sections from 56 cattle carcasses. Even though they used steers (n = 20), heifers (n = 16), and bulls (n = 16) carcasses, there were no significant effects of sex and its interaction with dissected and chemical fat content. Thus, their prediction equations were just based on pooled data.

In the validation performance, the RMSECV ranged between 1.82 and 2.03 chemical fat% units and the DEXA IMF predictions described between 95.4 and 96.4% of the variation in the pooled chemical fat data. The slight deviation of the slope from 1 and a bias means of 0% demonstrate good accuracy among pooled data and Angus groups (Table 5). The consistent

performance of DEXA predictions of chemical fat content among the validation groups ensures that DEXA technology can provide accurate IMF prediction. However, the Nellore group had an inferior accuracy performance based on a lower slope value, which ranged between 0.35 and 0.48 fat% units.

DEXA underestimation and overestimation of fat prediction among different studies may be due to the difference in DEXA equipment (Scholz et al., 2015) and sample type, bone presence or not (Lukaski et al., 1999). Also, the variation in the accuracy of fat prediction may be caused by the method used as the gold standard (chemical composition, dissected tissue weight, or computed tomography scanning) and differences in the fat content range of each particular study (Gardner et al., 2018). Nonetheless, DEXA scanning still provides an objective and non-invasive technology in the industry that can be used to optimize cut sorting before selling and in the research field, which could replace chemical analysis, considered the gold standard. Future studies are encouraged to evaluate DEXA IMF prediction in samples with a broader and more continuous range of IMF percentages to generate a robust regression model, regardless of fat content percentage.

4. Conclusion

This study demonstrates that DEXA can be used as a tool to predict chemical IMF content across beef *longissimus* steaks with a wide range of fat content. However, the precision and accuracy of fat prediction vary when DEXA performance is evaluated within low and high-fat content groups, demonstrating better performance in steaks with high-fat content.

Conflict of interest

The authors declare no conflict of interest.

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Tables and Figures

Table 1. Pearson correlations and *P*-values (in parentheses) across all samples between the parameters obtained from DEXA prediction and chemical analysis.

	DEXA prediction				*Chemical analysis*		
	Fat mass, g	Lean mass, g	Total tissue mass, g	Fat prediction, % of wet basis	Dry matter (%)	Fat content, % of dry basis	Fat content, % of wet basis
DEXA prediction							
Fat mass, g	1	0.126 (0.133)	**0.750 (<0.001)**	**0.977 (<0.001)**	**0.945 (<0.001)**	**0.946 (<0.001)**	**0.958 (<0.001)**
Lean mass, g		1	**0.751 (<0.001)**	-0.055 (0.516)	-0.041 (0.629)	-0.020 (0.810)	-0.048 (0.570)
Total tissue mass, g			1	**0.614 (<0.001)**	**0.601 (<0.001)**	**0.617 (<0.001)**	**0.606 (<0.001)**
Fat prediction, % of wet basis				1	**0.961 (<0.001)**	**0.964 (<0.001)**	**0.979 (<0.001)**
Chemical analysis							
Dry matter (%)					1	**0.909 (<0.001)**	**0.936 (<0.001)**
Fat content, % of dry basis						1	**0.990 (<0.001)**
Fat content, % of wet basis							1

*Significant correlations are highlighted in bold.

Table 2. Pearson correlations and *P*-values (in parentheses) for Angus crossbred steaks between the parameters obtained from DEXA prediction and the chemical analysis.

	DEXA predictions				*Chemical analysis*		
	Fat mass, g	Lean mass, g	Total tissue mass, g	Fat prediction, % of wet basis	Dry matter (%)	Fat content, % of dry basis	Fat content, % of wet basis
DEXA predictions							
Fat mass, g	1	0.037 (0.757)	**0.412** **(0.001)**	**0.782** **(<0.001)**	**0.679** **(<0.001)**	**0.521** **(<0.001)**	**0.670** **(<0.001)**
Lean mass, g		1	**0.926** **(<0.001)**	**-0.584** **(<0.001)**	**-0.393** **(0.001)**	**-0.476** **(<0.001)**	**-0.503** **(<0.001)**
Total tissue mass, g			1	**-0.237** **(0.045)**	-0.103 (0.390)	**-0.234** **(0.048)**	-0.204 (0.086)
Fat prediction, % of wet basis				1	**0.800** **(<0.001)**	**0.727** **(<0.001)**	**0.868** **(<0.001)**
Chemical analysis							
Dry matter (%)					1	**0.485** **(<0.001)**	**0.658** **(<0.001)**
Fat content, % of dry basis						1	**0.956** **(<0.001)**
Fat content, % of wet basis							1

*Significant correlations are highlighted in bold.

Table 3. Pearson correlations and *P*-values (in parentheses) for Nellore steaks between the parameters obtained from DEXA prediction and the chemical analysis.

	DEXA predictions				*Chemical analysis*		
	Fat mass, g	Lean mass, g	Total tissue mass, g	Fat prediction, % of wet basis	Dry matter (%)	Fat content, % of dry basis	Fat content, % of wet basis
DEXA predictions							
Fat mass, g	1	**0.358 (0.002)**	**0.445 (<0.001)**	**0.729 (<0.001)**	**0.376 (0.001)**	0.153 (0.199)	0.198 (0.096)
Lean mass, g		1	**0.995 (<0.001)**	**-0.352 (0.002)**	-0.190 (0.108)	**-0.257 (0.030)**	**-0.273 (0.020)**
Total tissue mass, g			1	**-0.260 (0.027)**	-0.146 (0.220)	-0.228 (0.054)	**-0.239 (0.043)**
Fat prediction, % of wet basis				1	**0.512 (<0.001)**	**0.333 (0.004)**	**0.390 (<0.001)**
Chemical analysis							
Dry matter (%)					1	-0.028 (0.817)	0.101 (0.397)
Fat content, % of dry basis						1	**0.990 (<0.001)**
Fat content, % of wet basis							1

*Significant correlations are highlighted in bold.

Table 4. Summary of data from steaks including mean ± standard error of the mean, minimum, maximum, and *P*-values comparing Angus and Nellore.

	Angus			Nellore			*P*-value
	Mean ± SEM	Minimum	Maximum	Mean ± SEM	Minimum	Maximum	
DEXA prediction							
Fat mass, g	55.61 ± 1.17	36.00	84.00	12.38 ± 0.27	8.00	19.00	<0.001
Lean mass, g	165.31 ± 2.79	111.00	232.00	160.50 ± 2.57	111.00	206.00	0.208
Total tissue mass, g	220.99 ± 3.08	154.00	288.00	172.86 ± 2.69	121.00	221.00	<0.001
Fat content, % of wet basis	25.29 ± 0.50	17.60	38.10	7.21 ± 0.15	4.40	10.40	<0.001
Chemical analysis							
Dry matter (%)	37.21 ± 0.49	17.00	47.24	24.37 ± 0.16	21.22	28.59	<0.001
Fat content, % of dry basis	56.33 ± 0.92	41.01	74.64	17.52 ± 0.54	9.78	29.27	<0.001
Fat content, % of wet basis	22.55 ± 0.56	14.05	36.82	4.59 ± 0.15	2.46	7.84	<0.001

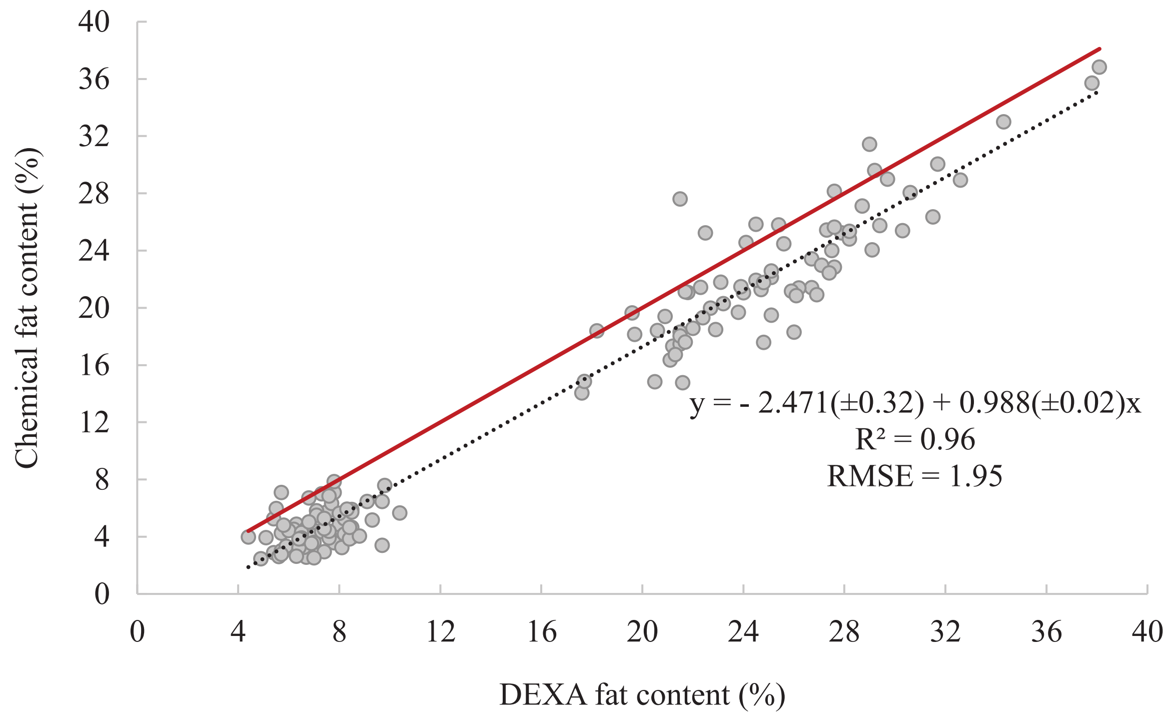

Figure 1. Linear regression between DEXA predicted fat content values on the x-axis and corresponding chemical analysis on the y-axis across all samples. Estimated parameters presented as mean ± standard error of the mean (SEM), coefficients of determination (R^2), and root mean square error (RMSECV) are depicted in the figure. The red line represents the perfect agreement between predicted and observed data with slope = 1 and intercept = 0.

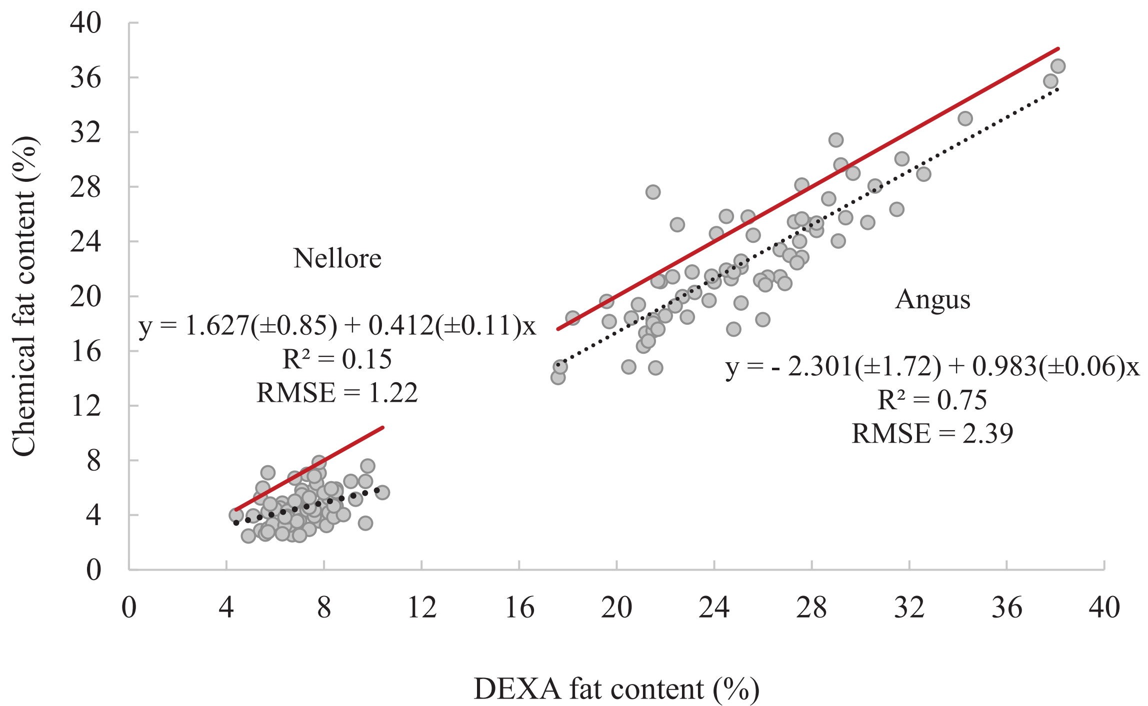

Figure 2. Linear regression between DEXA predicted fat content values on the x-axis and corresponding chemical analysis on the y-axis within Angus and Nellore groups. Estimated parameters presented as mean ± standard error of the mean (SEM), coefficients of determination (R^2), and root mean square error (RMSE) are depicted in the figure for both breeds Nellore and Angus. The red lines represent the perfect agreement between predicted and observed data with slope = 1 and intercept = 0.

Table 5. Accuracy and precision estimates for leave-one-out cross-validation of the model for DEXA predicting chemical fat% in *longissimus* steaks. For each group (pooled data, Angus and Nellore) models were validated within each of 5 data sets, with the mean, standard deviation, minimum, and maximum of these 5 tests being reported.

	Mean	Standard Deviation	Minimum	Maximum
Pooled data				
R^2	0.959	0.004	0.954	0.964
RMSECV	1.953	0.083	1.820	2.031
Slope	0.988	0.007	0.976	0.995
Bias	0.000	0.049	-0.066	0.070
Angus				
R^2	0.751	0.035	0.714	0.807
RMSECV	2.392	0.124	2.193	2.490
Slope	0.982	0.016	0.961	1.000
Bias	-0.001	0.113	-0.202	0.067
Nellore				
R^2	0.153	0.016	0.130	0.170
RMSECV	1.224	0.019	1.210	1.250
Slope	0.418	0.049	0.357	0.482
Bias	0.000	0.043	-0.066	0.042

RMSECV = root mean square error of cross validation.

Bias = represented by the difference between actual minus predicted value.

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CHAPTER 4

Genomic breed traceability of certified Angus beef of different brands sold in Brazil

ABSTRACT

Since the Brazilian beef herd is composed mainly of Nellore breed, we hypothesize that Nellore genetics significantly contributes to Angus-certified meat genetic composition. Therefore, the aim is to determine the extent to which the Nellore breed contributes to the genetic composition of certified Angus beef and evaluate the tissue composition of *longissimus* steaks from four different brands. DNA genotyping was performed in 261 samples of Angus-certified meat obtained from different commercial establishments in Brazil. Genomes of meat samples were then contrasted with reference samples, 72 samples of Angus, and 2052 samples of Nellore genotyped for 47,843 and 774,529 SNPs, respectively. After data quality control, we evaluated the genome profile of 96 meat samples, 71 genotyped Angus, and 1931 genotyped Nellore. Tissue composition and chemical analysis were assessed using DEXA and FoodScan^{TM} units, respectively. On average, meat samples presented 53% of Angus genome and 47% of Nellore genome. There was a tendency for a negative correlation between Nellore proportion and fat content (P = 0.065), indicating that an increase in Nellore genetics may lead to lower fat content. Evaluating the chemical analysis among the four brands, Brand 2 presented the highest collagen (2,06%) and fat (7.97%) content values compared to Brand 3, which presented the least amount of collagen (1.69%) and fat content (5.14%). In addition, Brand 1 differed from Brand 2 on protein content (P = 0.047), in which Brand 1 presented a higher value (21.39%). On DEXA composition, Brand 3 presented the least fat content (12.58%) compared to the others. Nellore genetics contributed up to 47% of the genome profile of Angus-certified beef evaluated in this

study. Also, there are differences in tissue composition between brands, with a tendency for leaner meat with a higher proportion of Nellore genetics.

Keywords: *Bos indicus*, *Bos taurus*, certified meat, fat, genotyping, traceability

1. Introduction

Meat certification and labeling expand the choice range of consumers concerned with animal products' origin, safety, composition profile, and quality (Bernués et al., 2003). Meat certification is possible due to traceability that tags the animal throughout the production chain from birth to slaughter and its products to the retailer (Baldo et al., 2010). Therefore, traceability gives consumers transparency and knowledge about meat products, whereas meat certification guarantees a certain degree of meat standards, making consumers more confident in their purchasing decisions.

The Brazilian Angus Association holds the Certified Angus Beef Program for its associates to provide a consistent and quality product for consumers. The Certified Angus Beef Program ensures product identity and quality following some specifications such as a minimum of 50% of Angus genetics with no excessive zebu influence, superior conformation, young animals, and at least a medium of fat cover classification (CNA, 2023). However, there is no genetic traceability program to ensure a breed composition of the animals. Angus selection is based on the phenotype evaluation of the black hide and absence of neck hump.

Genetic traceability identifies the product through DNA analysis, and it can be used as a reliable tool that allows animal and breed identification because DNA is stable and unalterable during animals' lifetime and throughout the supply chain, and it is presented in every single cell (Dalvit et al., 2007). Due to molecular advances, many single nucleotide polymorphisms

(SNPs) markers can be used to distinguish different animals and breeds using DNA analysis (Yang et al., 2013).

The Brazilian beef industry benefits from employing crossbreeding strategies that involves zebu and British breeds such as Angus because this crossbreeding program offer a combination of desirable traits, including improved adaptability, resistance to parasites, faster growth, and high-quality meat (Favero et al., 2019). Nellore is the leading cattle breed produced in Brazil. Therefore, certified Angus meat sold in Brazil can contain different proportions of Nellore breed due to commercial crossbreeding systems. Thus, we hypothesized that Nellore breed substantially contribute for the certified Angus beef sold in Brazil and that a higher Nellore proportion results in leaner meat and large variation on certified products. In this context, this study aimed to determine the extent to which the Nellore breed contributes to the genetic composition of certified Angus beef and evaluate the tissue composition of *longissimus* steaks from four different brands.

2. Material and Methods

Two hundred and sixty-one certified Angus steaks (*longissimus thoracis*) from four different brands were obtained from a commercial distributor in Minas Gerais state, Brazil. After 14 days aging period, all steaks were frozen at -20 ºC and stored at the Department of Animal Sciences of the Federal University of Viçosa, Minas Gerais, Brazil, until tissue extraction for DNA and chemical analysis.

Meat sampling and DNA extraction

A small sample portion of each steak was removed, avoiding adipose and connective tissue, and then macerated in liquid nitrogen. The pulverized samples were transferred to a 1.8 mL cryovial tube, identified, and stored in an ultra-freezer at -80ºC.

DNA extraction was performed in the Animal Biotechnology Laboratory of the Animal Science Department of the Universidade Federal de Viçosa, Minas Gerais, Brazil, using a DNA Extraction Kit from Thermo Fisher Scientific. The DNA extraction process was performed according to the extraction kit protocol (Pure Link™ Genomic DNA Mini Kit – K1820-01) from Thermo Fisher Scientific (Invitrogen Carlsbad, CA, USA). After obtaining the purified DNA, each sample's concentration and quality parameters were obtained using the Thermo Scientific™ NanoDrop Lite spectrophotometer.

DNA Genotyping

DNA genotyping of meat samples was conducted using the Illumina® GeneSeek Genomic Profiler Bovine 100K array. After data processing, three samples failed, totaling 258 genotyped samples. Illumina2preGS software from the BLUPF90 family (Aguilar, Misztal, Tsuruta, Legarra, & Wang, 2014) was used to convert the genotyped raw files from the steak samples, using a minimum GCscore of 0.6. As references, data from 72 genotyped Angus cattle for 47,843 SNPs and 2052 genotyped Nellore cattle for 774,529 SNPs were obtained, generating three evaluated groups: meat samples (MEAT), Angus (AN), and Nellore (NEL) (Figure 1).

Tissue composition and chemical analysis

Tissue composition was performed on entire frozen steaks (with the rib eye cap and without subcutaneous cover fat) using a medical DEXA unit (GE Healthcare, Lunar Prodigy Advance, USA), previously calibrated according to the manufacturer's recommendations at the Body Composition and Densitometry laboratory of the Federal University of Viçosa. Before scanning procedures, the Small Animal configuration mode of the GE Healthcare enCORE software, version 18, was selected. Also, average sample weight and dimensions (width x length

x height) were inputted into the software before scanning. The DEXA device provided results for fat tissue mass (g), lean tissue mass (g), total tissue mass (g), and fat content (%).

Also, the chemical composition of the steaks was evaluated by near-infrared analysis (AOAC method: 2007-04) on 100g of *longissimus thoracis* muscle ground without rib eye cap and fat cover, using the FoodScan^{TM} apparatus (FOSS, Hillerod, Denmark) (Figure 2).

Data analysis

The data from each group (MEAT, AN, and NEL) were submitted to quality control (QC) separately. Samples and SNPs with a call rate lower than 0.90, SNPs located in sex chromosomes and with no defined position, and duplicated samples were removed from the analysis. This resulted in 75,242 SNPs and 96 samples remained in the MEAT group, 34,586 SNPs and 71 samples remained in the AN group, and 743,665 SNPs and 1,931 samples remained in the NEL group for the genetic composition analysis. In total, 29,563 common SNPs remained for the analysis in the combined three databases. The SNPstats package (Clayton & Clayton, 2012), dplyr, and functions from the R software base package (https://www.r-project.org/) were used for QC.

Heterozygosity was also checked during QC on the X chromosome to determine the animal sex of the samples. Animals with heterozygosity below 0.20 was considered male and above 0.20 female. Of the 96 remaining MEAT samples, 18 were identified as male and removed from the dataset to avoid interaction between sex and tissue composition since there was no male within all brands.

The Admixture software (Alexander, Novembre, & Lange, 2009) was used to predict the genetic composition of all samples in the database, using a number of founder populations equal to 2 (K). The AGHmatrix package of the R software was used to construct a genomic

kinship matrix among all the evaluated animals, which was used in a principal component analysis (PCA) (Figure 3) to show the stratification of the evaluated populations.

The SAS 9.4 software (SAS – Statistical Analysis Systems Institute Inc., Cary, NC, USA) was used to perform the correlation analysis and the comparison between brands. Pearson correlations were determined using PROC CORR between Nellore proportion and chemical analysis across all samples. PROC MIXED procedure was used to evaluate the differences in chemical composition between brands. The least squares means (LSMEANS) were used to determine significant differences among treatments, establishing $P \leq 0.05$ as the significance level.

3. Results

Nellore genetics was present on the genome of all certified Angus steaks evaluated in this study (Figure 1). On average, meat samples presented 53% of Angus genome and 47% of Nellore genome, with proportions ranging from 28% to 72% of Angus genome. In addition, there was no difference on average in Nellore proportion when it was compared between certified Angus brands, with values ranging from 44% to 49% (Table 2).

As shown in Table 1, there was no significant correlation between the proportion of Nellore genetics and the chemical composition of the *longissimus* steaks. However, there was a negative correlation tendency between Nellore proportion and fat content ($P = 0.065$), indicating that an increase in Nellore genetics may lead to lower fat content.

There was a difference in dry matter, collagen, protein, and fat content between brands. Specifically, Brand 2 presented the highest collagen and fat content values compared to Brand 3, which presented the least amount of collagen and fat content (Table 2). In addition, Brand 1 differed ($P = 0.047$) from Brand 2 on protein content, presenting a higher value. Regarding DEXA composition, which included the rib eye cap and intermuscular fat, Brand 3 showed the

lowest fat content (12.58%) compared with the other brands. The lowest DEXA value of fat content on Brand 3 also supports the negative correlation between Nellore proportion and fat content.

4. Discussion

The Brazilian beef production is based on pasture, characterized by using Zebu breeds, especially Nellore, known for producing lean meat (Pereira et al., 2015). On the other hand, Angus cattle usually are prized for their marbled and tender meat compared to *Bos indicus* breeds (Wheeler et al., 1994). Also, because Angus breed presents reduced slaughter age and improved feed efficiency, it is preferably used in feedlot systems (Barcellos et al., 2017). In this study, there were no pure Angus animals among the evaluated meat samples, evidencing the contribution of the Nellore genetics to the production and quality of certified Angus meat in Brazil. Figure 3 illustrates the stratification of samples from the different groups evaluated using PCA, showing a clear separation between Nellore and Angus populations, with the evaluated meat samples clustering between the two groups.

Breed discrimination using genetic traceability allows the detection of mislabeling and protects the identity and value of a product (Moretti et al., 2022; Negrini et al., 2008). Evaluating the breed composition of Iberian ham, a traditional and regional product, via genetic traceability, García et al. (2006) reported that 20% of the ham samples were incompatible with the established regulation, as they did not attend to the minimum percentage of Iberian pigs' genome. Such studies underscore the importance of implementing a monitoring system based on molecular analysis to ensure the accuracy and quality of food products.

Although the meat samples evaluated in this study were certified as Angus, it is important to note that Nellore's genetics played a significant portion in their composition. The correlation tendency between fat content and Nellore proportion indicates that breed

composition may affect intramuscular fat. Our results showed differences in fat content between brands, which may be attributed to environmental factors such as diet and days on feed during the finishing phase since there was no difference in Nellore proportion between brands.

Intramuscular fat and collagen are derived from the same fibro-adipogenic progenitor cells. Thus, nutritional programs to enhance intramuscular fat can also increase intramuscular collagen deposition (Duarte et al., 2014), explaining the positive correlation between intramuscular fat and collagen content. However, despite a greater amount of collagen content, the solubility of intramuscular connective tissue is affected mainly by crosslinking abundance between collagen molecules (McCormick, 1994). Thus, fat content and collagen remodeling, which increases collagen solubility, are important factors that regulate meat tenderness (Fontes et al., 2021).

Apart from nutrition, the breed of cattle also plays a significant role in the potential of intramuscular fat deposition. Duarte et al. (2013) found a greater potential for marbling and connective tissue deposition in Wagyu cattle than Angus due to upregulation of early and late stage adipogenic markers. In agreement, Martins et al. (2015) suggested that the difference in intramuscular fat deposition between Angus and Nellore is due to a greater density of mesenchymal progenitor cells in Angus than in Nellore skeletal muscle.

According to Fernández et al. (2019), when the meat industry adds Angus advertising on a beef product label, its price tends to increase about 100% compared to no racial pattern certification on labels. According to Henchion et al. (2017), consumers are getting more concerned with credence attributes such as origin, animal welfare, and production system/feeding. If those extrinsic factors are trusted, consumers look for search attributes to evaluate alternatives and make selections such as price, certifications, and meat appearance. Lastly, eating quality as an experience attribute strongly influences future purchase decisions. Therefore, it is evident the importance of traceability and certifications along the meat chain

supply by promoting transparency, which is essential to get consumers' trusty and improve the overall production system.

5. Conclusion

In this study, Nellore genetics contributed up to 47% of the genome profile of Angus-certified beef sold in Brazil. Also, a negative correlation tendency was observed between Nellore proportion and fat content. Additionally, while there was no difference in Nellore proportion among brands, chemical composition varied.

Conflict of interest

The authors declare no conflict of interest.

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Tables and Figures

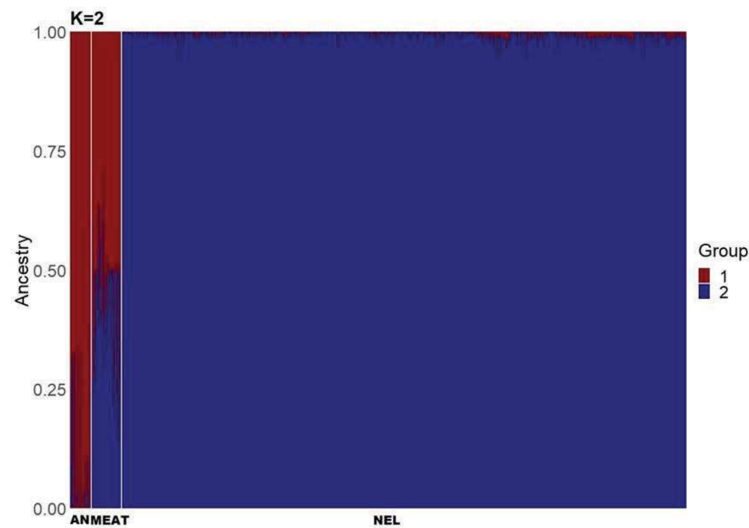

Figure 1. Genome proportion of Angus and Nellore breed in the evaluated groups (Angus – AN; Nellore - NEL; Meat samples – MEAT). Each vertical bar represents one sample, in which the red bar represents the proportion of Angus ancestor genome (Group 1), and the blue bar represents the Nellore ancestor genome (Group 2).

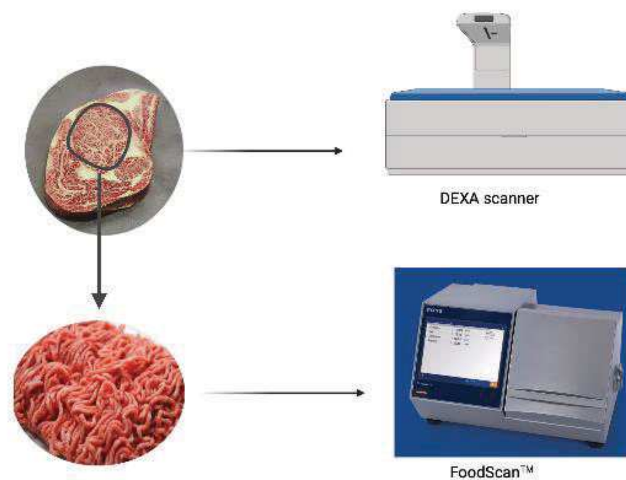

Figure 2. The entire ancho steak was used on DEXA scan while ground *longissimus* was used for chemical analysis.

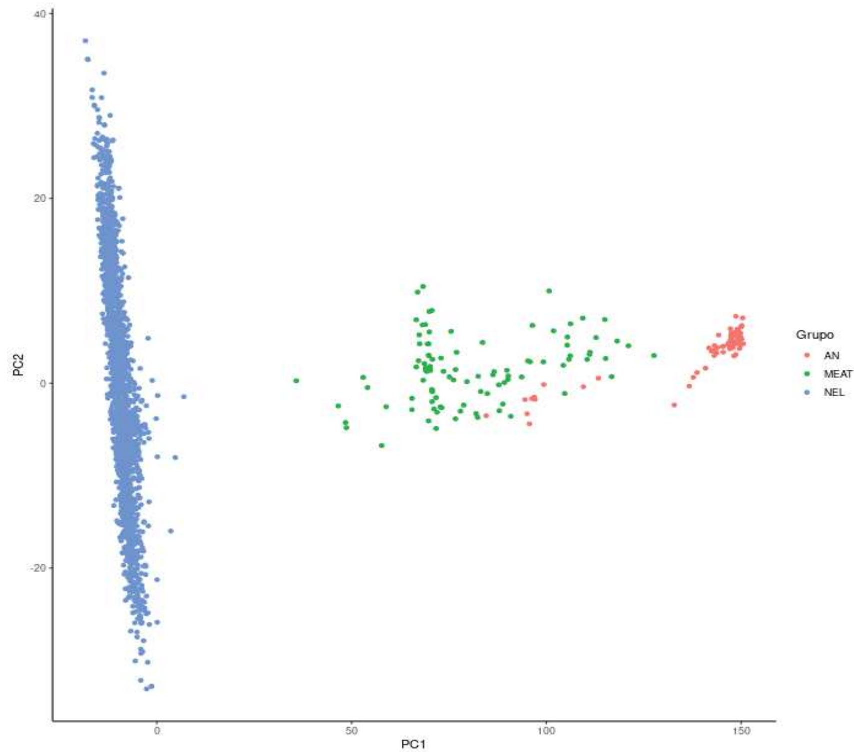

Figure 3. Meat samples distribution according to the first two principal components analyses (PC1 and PC2)

Table 1. Pearson correlations and *P*-values (in parentheses) among all data between the Nellore proportion and chemical composition.

	Nellore proportion	Dry matter	Collagen	Protein	Fat	Ash
Nellore Prop.	1	-0.170	-0.161	0.201	-0.210	0.098
		(0.1374)	(0.160)	(0.077)	(0.065)	(0.394)
Dry matter		1	0.736	-0.623	0.964	0.056
			(<0.001)	(<0.001)	(<0.001)	(0.625)
Collagen			1	-0.466	0.779	-0.379
				(<0.001)	(<0.001)	(0.001)
Protein				1	-0.766	-0.161
					(<0.001)	(0.159)
Fat					1	-0.069
						(0.550)
Ash						1

Table 2. Chemical characteristics of the *longissimus* thoracis muscle between the four different certified Angus brands.

Sample features (%)	Certified Angus Brands				SEM	*P*-value
	Brand 1 (n = 16)	Brand 2 (n = 37)	Brand 3 (n = 10)	Brand 4 (n=15)		
Nellore proportion	0.492	0.454	0.442	0.497	0.009	0.1517
Dry matter	31.202ab	31.860a	29.407b	30.439ab	0.253	0.008
Collagen	1.921ab	2.062a	1.698b	1.895ab	0.042	0.0325
Protein	21.390a	20.741b	21.282ab	20.889ab	0.098	0.0473
Fat	6.712ab	7.959a	5.140b	6.547ab	0.323	0.0268
Ash	3.103	3.164	2.990	3.005	0.052	0.5976

SEM = Standard error mean.

Means within the same row without common letters differ ($P \leq 0.05$).

Table 3. DEXA composition of the *longissimus* steak with the rib eye cap.

Sample features	Certified Angus Brands				SEM	*P*-value
	Brand 1 (n = 16)	Brand 2 (n = 37)	Brand 3 (n = 10)	Brand 4 (n=15)		
Total tissue (g)	289.38c	226.19d	340.10b	365.12a	7.291	<0.001
Lean tissue percentage (%)	77.06b	75.65b	87.42a	74.97b	0.601	<0.001
Fat percentage (%)	22.94a	24.27a	12.58b	25.04a	0.602	<0.001