Does transport stress have any effect on carcass quality of Nellore cattle (Bos taurus indicus) in Brazil? - A case study

Nathalia da Silva Rodrigues Mendes, Renato Rodrigues Silva, Tatianne Ferreira de Oliveira, Marie-Pierre Ellies-Oury, Jean-François Hocquette, Sghaier Chriki

Translational Animal Science, 2024, **8**, txad134
https://doi.org/10.1093/tas/txad134
Advance access publication 8 December 2023
Meat Science

OXFORD

AMERICAN SOCIETY OF ANIMAL SCIENCE

Does transport stress have any effect on carcass quality of Nellore cattle (*Bos taurus indicus*) in Brazil? — A case study

Nathalia da Silva Rodrigues Mendes[†,‡], Renato Rodrigues Silva[||], Tatianne Ferreira de Oliveira[†], Marie-Pierre Ellies-Oury[‡,$], Jean-François Hocquette[‡], Sghaier Chriki[‡,¶,1,]

[†]Department of Food Science and Technology, School of Agronomy, Federal University of Goiás-UFG, Campus Samambaia, CEP 74690-900, Goiânia, Brazil
[‡]Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE), Université Clermont Auvergne, VetAgro Sup, UMR Herbivores, UMR1213, Recherches sur les Herbivores, Theix, 63122 Saint-Genès Champanelle, France
[||]Department of Institute of Mathematics and Statistics, Federal University of Goiás, Goiânia, Brazil
[$]Department of Feed and Food, Bordeaux Sciences Agro, CS 40201, 33175 Gradignan, France
[¶]ISARA, CEDEX 07, 69364 Lyon, France
[1]Corresponding author: schriki@isara.fr

Abstract

With the increasing concomitant demands for Brazilian beef and in particular high-quality beef, there is a need for observational studies of the effects of pre- and post-slaughter practices on beef carcass traits. We hypothesized in our case study that pre-slaughter transport of bovines over significant distances would induce stress in animals, and that this would reduce carcass quality because of higher pH resulting from long-distance transportation. To test this hypothesis, 30,230 Nellore carcasses from a private slaughterhouse were evaluated 24 h *postmortem*. Analysis showed correlations between animal maturity, ultimate pH, distance, and carcass weight. More precisely, there was a slight positive correlation between ultimate pH and weight (but not with transportation distance) and a slight positive correlation between maturity and weight. A linear regression model ($R^2 = 0.016$) failed to show distance having a significant effect on ultimate pH ($P = 0.63$), while carcass weight significantly affected ultimate pH ($P < 0.001$) with a low coefficient of 0.0003. Maturity negatively affected ultimate pH also ($P < 0.001$) but with also a small effect (−0.0008). Results (from 95% confidence intervals of variance of the random effects and of the random errors) showed that the variability within farms was higher than between farms. The linear mixed model showed that maturity had a significant effect on carcass weight value ($P < 0.001$) with a large coefficient of 2.90. The R^2 of the linear mixed model was 46.03%. In conclusion, while weight and maturity both affect ultimate pH, long distances did not significantly impact ultimate pH and therefore the beef quality from Nellore cattle. This could be because of low stress during transport, as well as the physical characteristics of the Nellore breed that favor greater resistance to tropical climatic conditions.

Lay Summary

With the increasing concomitant demands for Brazilian beef and for high-quality beef, there is a need for observational studies of the effects of pre- and post-slaughter practices on beef carcasses. In our case study, we hypothesized that pre-slaughter transport of bovines over significant distances would induce stress in animals and that this stress would reduce carcass quality because of the higher pH which would result from long-distance transportation. To test this hypothesis, 30,230 Nellore carcasses from a private slaughterhouse were evaluated 24 h *postmortem*. Analysis showed correlations between animal maturity, ultimate pH, distance, and carcass weight. More precisely, there was a slight positive correlation between ultimate pH and weight (but not with transportation distance) and a slight positive correlation between maturity and weight. Statistical analysis showed that the variability within farms was higher than between farms. In conclusion, while carcass weight and maturity both affect ultimate pH, long distances did not significantly affect ultimate pH, and therefore had no significant effect on the quality of beef from Nellore cattle. This could be because of low stress during transport, as well as the physical characteristics of the Nellore breed that favor greater resistance to tropical climatic conditions.

Key words: animal stress, beef, carcass traits, eating quality

Introduction

Beef eating quality is an intrinsic quality trait, which depends on both pre- and post-slaughter factors (Liu et al., 2022; Pogorzelski et al., 2022). The main attributes used to characterize beef eating quality are flavor, juiciness, tenderness, and overall liking. These descriptors appear to be highly variable and dependent on many interacting factors that are difficult to manage, such as the *antemortem* properties (breed, age, maturity, carcass fat level, fatty acid composition of cuts, etc.) and the *postmortem* elements (slaughter processes, such as carcass handling, aging, and storage (Devlin et al., 2017)). Prior to slaughter, animals are exposed to situations that can trigger stress responses, which can reduce the sensory quality of the meat (Gruber et al., 2010).

An observational case study would be able to determine whether transport time might be a significant source of stress. For the purposes of this paper, transport time means the total time that an animal is confined in a vehicle without food,

Received April 26, 2023 Accepted December 7, 2023.

water, or rest to transport it from the farm to the slaughterhouse. This includes time spent waiting to depart after loading, time in transit, and stationary periods, as well as waiting to off-load (Schwartzkopf-Genswein et al., 2012). Transport time can be significant in large countries such as Brazil, where livestock are often transported long distances from farm to slaughterhouse, which may be located in another part of the country. Brazilian legislation, Normative Instruction 09/2021, allows a maximum of 12 h continuous transportation before requiring a rest stop for food and water (Brazil, 2021). Improper handling during transport and at the slaughterhouse can lead to muscle glycogen depletion, inadequate acidification, and high ultimate pH. This results in darker meat, with lower sensorial properties, especially tenderness (Gruber et al., 2010), juiciness, and flavor (Hemsworth et al., 2011). For example, it is well known that an ultimate pH higher than 6 between 12 and 48 h *postmortem* results in dark meat cuts (a defect known as dark, firm, and dry [**DFD**]) with a shorter shelf life because of increased susceptibility to microbial contamination (Pérez et al., 2013). However, most studies of the effects of transport time on beef quality have been conducted using *Bos taurus taurus* cattle reared and transported in systems different from those used in Brazil (Maria et al 2003; Gonzalez et al., 2012 a,b; Chulayo et al., 2016; Polkinghorne et al., 2018), where zebu cattle, which are characterized by a more reactive temperament, predominate (Cooke et al., 2020). Zebu beef is typically less tender with less marbling than that of taurine cattle (*Bos taurus taurus*), particularly when compared to the Angus breed (Seideman et al., 1982; De Andrade et al., 2020).

Information on the effects of transport times on the quality of meat from zebu cattle is still scarce, as there have only been a limited number of studies of these animals, and moreover, only in the northern and southern regions of Brazil (Ferreira et al., 2006; Mendonça et al., 2018; Lacerda et al., 2022). Even though the central-western region is unquestionably important for the Brazilian beef industry (ABIEC, 2022) studies of the effects of transport time in this vitally important region on beef quality are lacking.

We hypothesized that pre-slaughter transport of bovines causes stress to the animals, and results in carcasses of lower quality with a higher final pH because of that stress. Consequently, the aim of this observational case study was to investigate the effects of stress during transport between farm and slaughterhouse, specifically on ultimate pH for Nellore cattle, while controlling for hot carcass weight and maturity.

Material and Methods

Institutional Animal Care and Use Committee (IACUC)

This slaughterhouse is regulated by the Brazilian Federal Inspection Service (S.I.F 2872).

Article 110 of the Regulamento Industrial e Sanitário de Produtos de Origem Animal - RIISPOA (Brazilian Industrial and Sanitary Regulation of Animal Products), requires that cattle be given a rest period and water. Fasting in the slaughterhouse pens for 24 h prior to slaughter. Pre-harvest handling conformed to good animal welfare practices with slaughter procedures following the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 2004, 2021). These documents are the Animal Movement Permit and the farmer's declaration of having followed the standard and expected protocols (Brasil, 2021). Slaughter conditions conformed to traditional or Halal procedures (CIBALLAHAL, 2022).

Dataset

We used a data set provided by a private company located in Inhumas (Goiás, Brazil) (49°28′14.84′′W and 16°20′31.42′′S). This slaughterhouse can process up to 700 cattle per day (80 to 90 animals/h) and is regulated by the Federal Inspection Service (S.I.F. 2872). The climate in this region is tropical with an average annual temperature of 23.1 °C with an average rainfall of 1,516 mm with a Heat Index in Inhumas of 22% (Brasil, 2022).

The rainy season is frequently cloudy and overcast; the dry season is almost always cloudless. Throughout the year, temperatures generally range from 16 to 32 °C and rarely fall below 13 °C or above 36 °C (Brasil, 2022).

The company purchases animals from different areas of the state of Goiás, and maintains them within a production system typical of this region, i.e., a pasture-based tropical conditions system. These animals are representative of the beef cattle reared in central-western Brazil. Pre-harvest handling conformed to good animal welfare practices and slaughter procedures and followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 2004) and Technical Regulations for Pre-slaughter Management and Humane Slaughter and the stun methods authorized by the Ministry of Agriculture, Livestock and Supply (BRASIL, 2021).

The data set for the period from January to August 2021 has 35,126 records of beef carcass traits such as breed (Nellore or Crossbreed), category (Bull, Cow, Dutch bull, Dutch cow, Heifer, Marruco ox, Taurus), conformity, maturity (age), carcass weight, and ultimate pH. Categories are based on age and sex and can be further classified into calves or heifers, heifers, or bullocks, which in turn may be castrated or whole, as well as cows or bulls for slaughter. Conformity refers to the standards to which this meat should be processed for the market for which it is intended. During the period in which this study was conducted, the meat followed the standards established for the following countries: Brazil, China, and Halal consumers' countries (Iran and Saudi Arabia) Central Islâmica Brasileira De Alimentos Halal (CIBALLAHAL, 2022).

Maturity (age) was determined by dentition: milk teeth (up to 20 mo), two teeth (21 to 24 mo), four teeth (25 to 30 mo), six teeth (31 to 41 mo), and eight teeth (42 mo for above). Following the post-harvest and head inspection, the number of permanent incisors was recorded for each Nellore bull. A pair of teeth was considered to be present when either tooth of a pair had penetrated the gum. Carcass weight refers to the edible portion after slaughter, which is composed of meat, fat, and bones, and is measured in kilograms (kg). The ultimate pH was measured with a pH-meter (AK103 - Akso Produtos Eletronicos Ltda) in the *Longissimus thoracis* muscle at 13^{th} rib of the left half of the carcass at parallel points in the same section, and 24 h post-slaughter. With regard to carcass temperature and amb,ient temperature, the slaughterhouse maturation system varies according to market: Chile requires 2 to 4 °C for 24 h from 10 °C ambient, while Uruguay and other countries require 4 °C for 24 h from 10 °C ambient. Brazil's standards for carcass temperature consist of a minimum of 2 °C and a maximum of 5 °C 24 h after slaughter from 10 °C ambient.

In addition, animals were evaluated according to fat finishing, which was performed visually using a 9-point scale with scores from 1 to 9 (1 - absent fat; 2,3,4 - scarce; 5,6,7 - medium; 8 - uniform, and 9 - excessive fat). The distance from farm to slaughterhouse is given in kilometers, with each 100 km corresponding to 1 h 30 min driving time.

The numbers of the different modalities of the variables are shown in Table 1. We started with 35,126 records of beef carcasses. Of these, 99.28% were Nellore breed, 97.18% bulls non-castrated, 98.99% Halal, and 89.14% fat finishing. Restricting our target population to Nellore bulls with Halal conformity and rare fat finishing, meaning 30,230 carcasses, reduced the number of potential confounding variables (Table 1). Because of the large sample size, each of the variables studied can have significant effects, but this was minimized by focusing on ultimate pH and the variables of interest, i.e., a distance between farm and slaughterhouse, carcass weight, and maturity. For this reason, our sample is limited to ultimate pH, distance from farm to slaughterhouse, carcass weight and maturity as shown in Table 2.

Table 1. Profiles of carcasses based on breed, category, fat finishing and conformity

Parameter	Value	*n*[1]	%
Breed	Crossbreed	253	0.72
	Nellore	34,873	99.28
Category	Bull	34,135	97.18
	Cow	610	1.74
	Dutch bull	79	0.23
	Dutch cow	8	0.02
	Heifer	173	0.49
	Marruco ox	48	0.14
	Taurus	73	0.21
Fat finishing	Excessive	6	0.08
	Medium	3,748	10.67
	Rare	31,311	89.14
	Uniform	61	0.17
Conformity[2]	Brazil	1	0.003
	China	350	1.00
	Halal	34,775	99

[1]Number of carcasses.
[2]The standards according to which this meat should be processed for its intended market.

Slaughter Process and Conditions

The company follows regularly inspected procedures for slaughtering animals (Brasil, 2004). Animals are processed for slaughter only after documents accompanying the lot are inspected. These are the Animal Movement Permit and the farmer's declaration of having followed the standard and expected protocols. Both drivers and stockyard staff have been trained in animal welfare. Animal lots are separated by origin. After unloading, the livestock trucks are washed and disinfected. The animals rest and are given water for at least 6 h before slaughtering. Animals approved for slaughter pass through a spray bath of pressurized hyper-chlorinated water. Slaughter conditions are conformed to traditional or Halal procedures. After this, an assistant from the Inspection Service determines the age of the animals using dentition and then stamps the carcass with its age group. The half-carcasses are graded for fat finishing, absence or presence of bruising, sex, and weight. After the half-carcasses are stamped by the Federal Inspection Service, they are weighed and traceability labels are applied to the hindquarter, forequarter, and flank. The half-carcasses are washed with jets of pressurized water to remove bone fragments and blood clots, then, they are placed in chillers suspended by the Achilles tendon at +4 ± 1 °C to undergo maturation after a pH drop. After chilling, the half-carcasses are classified by ultimate pH (see section "Institutional Animal Care and Use Committee (IACUC)").

Statistical Analysis

The data were analyzed using R software (version 4.1.2 - R Core Team, 2022). After descriptive analysis, a box plot according to DuToit et al. (2012). Median values are indicated by the line within the box plot. The box extends from the 25th to 75th percentiles and whiskers indicate the minimum and maximum values. Analysis of Variance (ANOVA) was performed using the aov() function to determine the significant differences between carcass characteristics.

Pearson correlation was calculated with the ggplot2 package using the "pairs.panels" function.

Principal Component Analysis (PCA) was performed using the package "FactoMineR" to represent and model multidimensional point cloud datasets, showing whether relationships exist between the studied variables. PCA allows for the calculation of new variables, called principal components, which capture the variability in the data. This enables information to be described with fewer variables than originally present. The principal components are linear combinations of the original variables. The first principal component is the combination of variables that explains the greatest amount of variability in the data. The second and subsequent principal components describe the maximum amount of remaining variability and

Table 2. Raw values for mean, standard deviation (SD), coefficient of variation (CV), minimum, and maximum for the measured traits for our studied population of 30,230 carcasses

	Mean	SD	CV (%)	Minimum	Maximum
Ultimate pH	5.75	0.07	1.22	5.0	6.04
Distance, km	203	115.59	57.44	10	547
Time, h	3	1.73	57.44	0.15	8
Carcass weight, kg	298.9	34.14	11.41	150.5	553
Maturity, months	203	1.97	48.47	0	8

must be independent (orthogonal) between them and to the first principal component (Husson et al., 2016).

Multivariate Regression Models were developed by using the “lm” (linear model) function and “lm.beta” R Package (Behrendt, 2014). The linear regression models were carried out to study the relationship between pH and explanatory variables such as carcass weight, maturity (age), and distances as follows:

$$y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \beta_3 x_{i3} + \epsilon_i,$$

where y_i represents the ith measured pH on the beef carcass, β_0 is the intercept, $\beta_1, \beta_2, \beta_3$ are the regression coefficients, x_{i1} represents the ith carcass weight, x_{i2} represents the physiology animal maturity evaluated on carcass (this parameter being highly correlated to chronological age), x_{i3} represents the ith farm and ϵ_i the random error.

We fitted a mixed model to analyze if the carcass pH variance within the farms was higher than between the farms. Mixed-effect models are used to describe relationships between a response variable and some of the covariates in data when grouped according to one or more classification factors. Examples of such grouped data include longitudinal data, repeated measures data, multilevel data, and block designs. By associating common random effects with observations sharing the same level of a classification factor, mixed effects by grouping the data by grouping the data models flexibly represent the covariance structure induced by the grouping of the data (Pinheiro and Bates, 2000).

The statistical model can be expressed as follows:

$$y_{ij} = \mu + b_i + \epsilon_{ij},$$

Where y_{ij} represents the ijth pH measured on i^{th} farm and j^{th} carcass, μ is the intercept, b_i is the random effect for i^{th} farm and ϵ_{ij} the random error.

Additionally, we fitted a mixed to model the carcass weight in function of maturity using the farm as a random effect. The model was the following:

$$y_{ij} = \beta_0 + \beta_1 x_i + b_i + \epsilon_{ij},$$

where y_{ij} represents the ij^{th} weight measured on i^{th} farm and j^{th} carcass, β_0 is the intercept, β_1 is the straight slope, x_i represents the maturity (age) evaluated on i^{th} farm, b_i is the random effect for i^{th} farm and ϵ_{ij} the random error. Confidence intervals for the random effect variance represent the ultimate pH variability between farms, and confidence intervals for random error represent the ultimate pH variability within farms. For this model, the likelihood ratio test was performed to assess the significance of the random effect in the model. The value of R^2 was calculated using the rsq library. All computations related to fitting mixed models were done using the nlme R package (Pinheiro and Bates, 2022).

Results and Discussion

Descriptive Analysis

The pH distribution is left-skewed and has low variability (Figure 1a), with a coefficient of variation of 1.22% (Table 2) and an interquartile range less than 0.1. It can be seen that the median value is 5.75 and therefore 50% of the samples have pH values less than 5.75. The first quartile 25% is for has an ultimate pH value of less than 5.71, and the third quartile 75% has is for ultimate pH values higher than 5.79. The box plot shows many outliers which are easily detectable which contrasts with the low sample variability of ultimate pH (Figure 1b). This may be because of technical problems (pH-meter calibration, for instance), operator error, biological problems in specific carcasses, and so on. It is worth noting that the removal of outliers does not significantly affect these results, given the large sample size.

The ultimate pH range in this study agrees with the available literature (Pérez et al., 2013) and as required by the two markets (Iran and Saudi Arabia) to which the company exports beef with Halal conformity. Iran allows a maximum ultimate pH of 6.2 as specified by its regulatory agency, and Saudi Arabia allows a maximum ultimate pH of 6.0 as specified by its regulatory agency. The ultimate pH averaged 5.74 ± 0.07 which is consistent with the findings of Silva et al. (2019), whose values were 5.78 ± 0.01, where these authors evaluated the effect of castration on the carcass and meat quality traits of Nellore cattle. Our results also fit with those of Lacerda et al. (2022), where the ultimate pH mean was 5.82 ± 0.11.

In our data, 22.30% of the total number of carcasses had an ultimate pH higher than 5.8 and lower than 6.04 (the maximum pH value in this study). We also emphasize that an ultimate pH higher than 5.8 is considered DFD meat by some authors, since these changes in final pH cause considerable losses for the beef sector, as the most frequent problems caused by stress in cattle are weight loss, carcass lesions and altered meat quality, mainly due to increased pH (>5.8), affecting tenderness and meat color (dark meat) (Lomiwes et al., 2014; Hughes et al., 2017).

Pérez et al. (2013) consider meat to be DFD with an ultimate pH higher than 6 between 12 and 48 h *postmortem* to result in dark meat cuts with a shorter shelf life because of increased susceptibility to microbial contamination. In our data, only 0.023% of the total number of carcasses had an ultimate pH higher than 6.0.

We chose to compare our pH results with the literature, considering we did not analyze meat color, shear force, etc. The ultimate pH range in this study was acceptable to the two markets (Iran and Saudi Arabia) to which the company exports beef with Halal conformity. Iran allows a maximum ultimate pH of 6.2 as specified by its regulatory agency; and Saudi Arabia allows a maximum ultimate pH of 6.0 as specified by its regulatory agency, while Brazil allows a maximum ultimate pH of 6.0 as specified by its regulatory agency.

These ultimate pH values can be explained by 1) the fact that animals were raised using a pasture system, which leads to higher ultimate pH values than grain-fed cattle (Apaoblaza et al., 2020) and 2) the use of non-castrated males, which can lead to increased stress, because castrated animals are easier to handle than non-castrated animals, since castration results in changes in behavior, namely in becoming calmer, gentler, more obedient, and more amenable to management (Duarte et al., 2011). These two reasons would justify the abnormal ultimate pH in some evaluated carcasses but are insignificant when compared to the effects of road transport time on ultimate pH as considered in this study (Figure 1a and b).

The distribution of distances between farm and slaughterhouse is left-skewed with a high variability (Figures 2a and b), with a coefficient of variation of 57.44% (Table 2). The

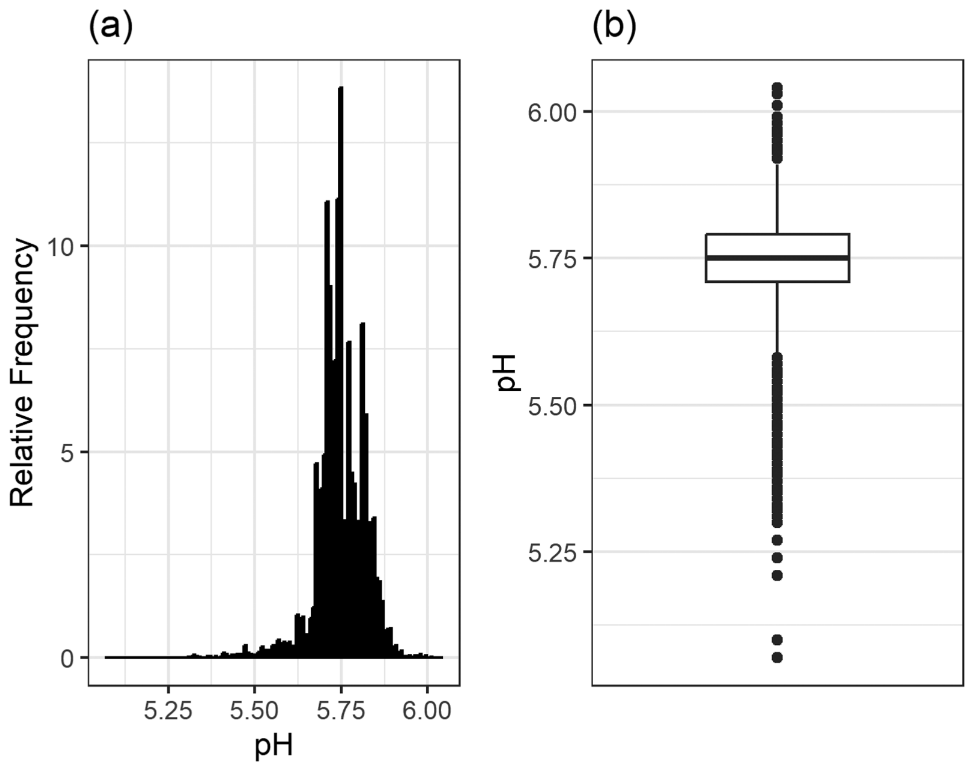

Figure 1. Frequency distribution of ultimate pH values in beef carcasses, represented as a histogram (a) and box plot (b).

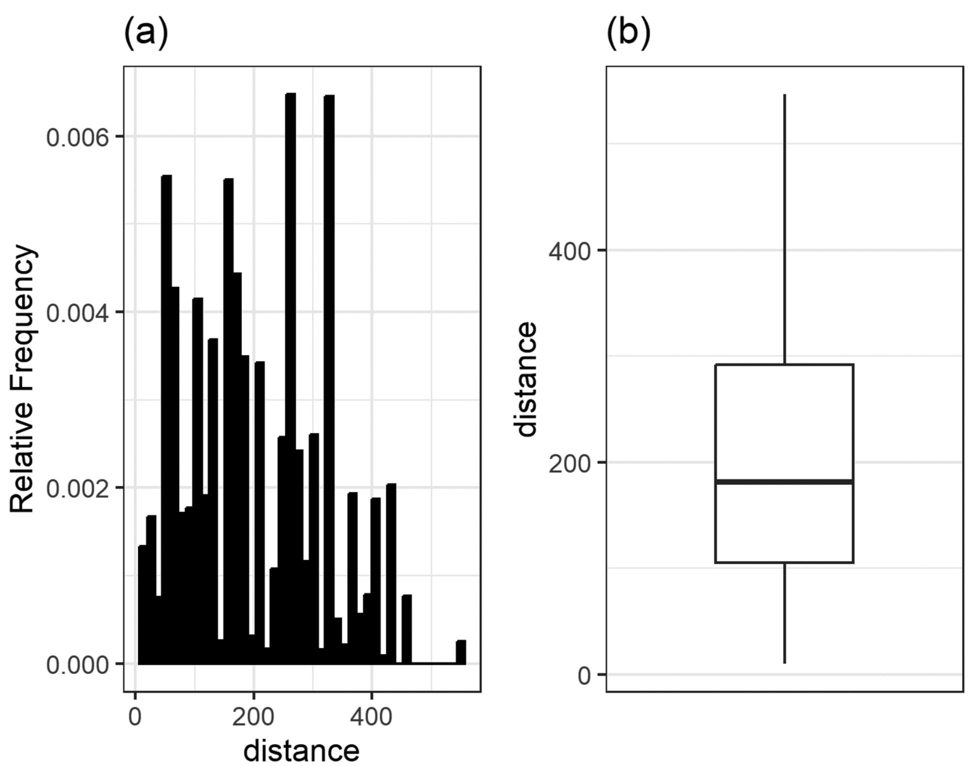

Figure 2. Frequency distribution of distance between farm and slaughterhouse (km) in beef carcasses, represented as a histogram (a) and box plot (b).

median value is 181 km and the first quartile 25% with a distance value of less than 105 km and the third quartile 75% has a distance value of more than 292 km. It should be noted that the slaughterhouse in this study was located near the geographic center of the Goiás state, and that the average distance was not as great as those reported in Chile (Werner et al., 2013) or Canada (Gonzalez et al., 2012 a,b; Warren et al., 2010), which had an average distance greater than 400 km. However, the distances reported for the present study are similar to those in studies conducted in the northern and

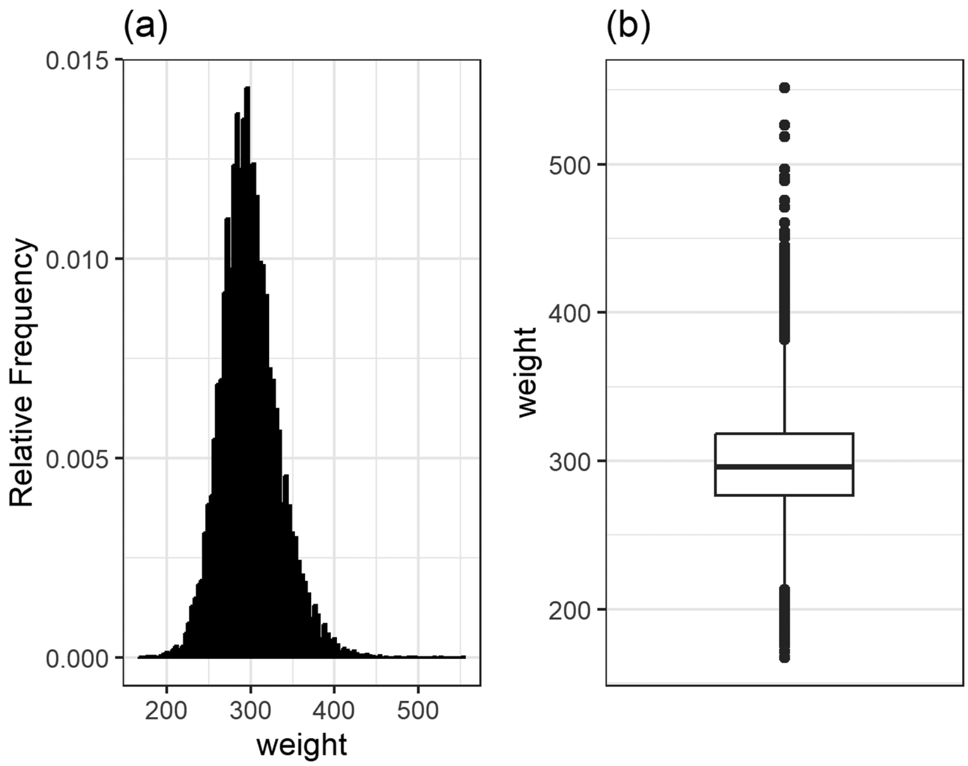

Figure 3. Frequency distribution of hot carcass weight (kg) in beef carcasses, represented as a histogram (a) and box plot (b).

southern regions of Brazil (Mendonça et al., 2018; Lacerda et al., 2022), and are in accordance with Brazilian legislation, Normative Instruction 09/2021, which allows a maximum of 12 h of continuous transport before requiring a rest stop for food and water (Brasil, 2021).

Although animal transport of long duration is more likely to compromise animal welfare than that of shorter duration, it is important to recognize that it is not the duration of the journey per se, but the associated negative aspects, i.e., lack of food, lack of water and high temperature, which are the source of any detrimental effects. Stress from extreme temperatures and lack of food, water, and rest, for example, are all exacerbated by length of exposure, i.e., journey duration (Nielsen et al., 2011). Furthermore, once animals have adapted to their new situation, distance is a relatively minor problem when compared to loading densities, vehicle design, road conditions, or driver skill (Strappini et al., 2009). In this study, these factors were controlled for, and the distance had no significant influence on beef quality, as shown by the ultimate pH values.

The weight distribution is left-skewed and has high variability (Figure 3a), with a coefficient of variation of 11.41%. The median value is 296.50 kg with the first quartile 25% having a weight value of less than 275.50 kg and the third quartile 75% with a weight value of more than 319.50 kg. The many outliers shown in the box plot (Figure 3b) can be explained by different ages. This agrees with the results of Bureš and Bartoň (2012) who evaluated the effects of gender and age at slaughter on growth, feed intake, carcass composition, and meat quality of *musculus longissimus lumborum* attributes in Charolais × Simmental. They found that bulls slaughtered at 14 mo of age were lighter than those slaughtered at 18 mo of age at the end of the fattening period. This same result in Nellore bulls was also observed by Silva et al. (2019). The average hot carcass weight in the present study was 298.90 ± 34.14 kg and which agrees with the value of 248 ± 34.20 kg observed for Nellore bulls by Mello et al. (2018) and with 236.60 kg found by Silva et al. (2019).

The maturity distribution is left-skewed and has high variability (Figure 4a), with a coefficient of variation of 48.47%. The median is 4 teeth and the first quartile 25% with a maturity value of less than 2 teeth and the third quartile 75% has a value of more than 4 teeth. The box plot indicates many outliers (Figure 4b). This is due to the sample size and in the present study, the experimental design did not stipulate the age range of the animals studied since the objective was to make an exploratory analysis of the company's data. This agrees with results from Duarte et al. (2011) with most of the animals with 4 permanent incisors teeth and when evaluating the physical and chemical characteristics of meat from Nellore bulls with 4 permanent incisors teeth he found acceptable results for tenderness.

Correlations Between Beef Carcass Traits

The matrix correlation between beef carcasses shows weak correlations between traits and no relationship between the two variables of interest namely ultimate pH and distance (Table 3).

There was no significant correlation between ultimate pH and distance ($r = 0.01$) on the one hand and between ultimate pH and maturity ($r = 0.01$) on the other hand. In the conditions studied, the hypothesis that long distances would significantly interfere with beef quality was not confirmed for the Nellore breed. In this study, the transportation distance and time may have been insufficient to cause enough stress to the animals to deplete glycogen stores. A longer transport time (14 h) may result in changes to the rumen environment, leading to an increased acetate/ propionate ratio. This would

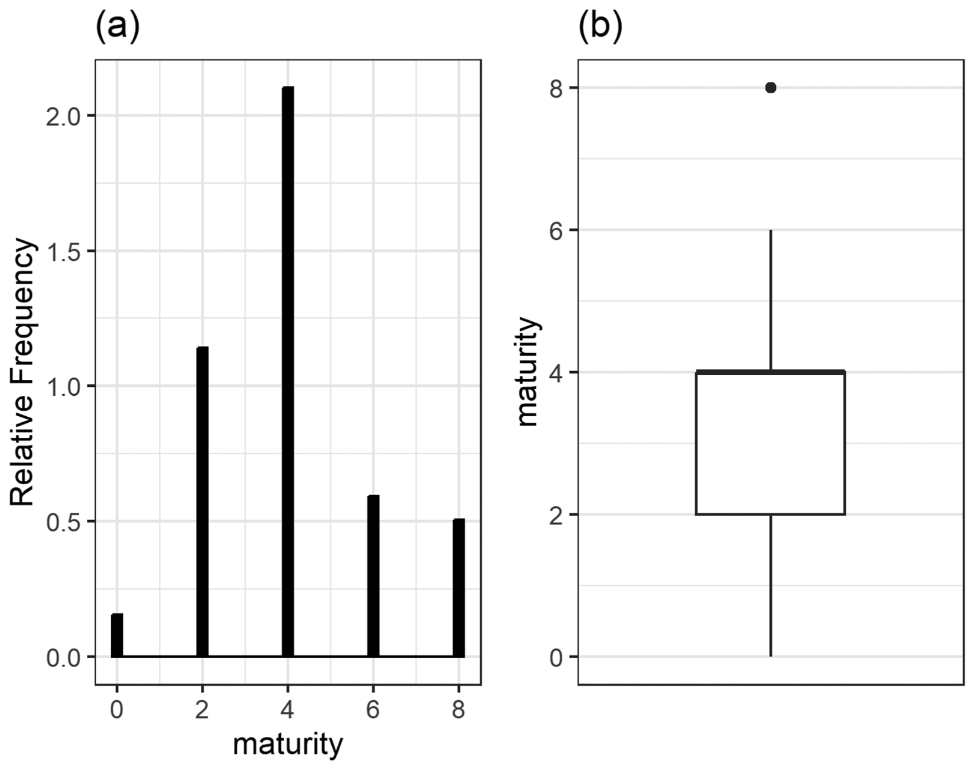

Figure 4. Frequency distribution of maturity (age) in beef carcasses, represented as a histogram (a) and box plot (b).

Table 3. Correlation coefficients between beef carcass traits

	pH	Distance	Weight	Maturity
Ultimate pH	1	0.01[1]	0.12[2]	0.007[1]
Distance, km	—	1	0.05[2]	−0.03[2]
Carcass Weight, kg	—	—	1	0.23[2]
Maturity, months	—	—	—	1

[1]Correlation is not significantly different ($P > 0.05$)
[2]Correlation is significantly different at 0.01 ($P < 0.01$).

reduce the quantity of circulating glucose and affect ultimate pH (Deng et al., 2017). This probably did not occur in the animals studied because even the longest time (8 h) did not impede the decline in ultimate pH.

Another factor may be due to the physical characteristics of the Nellore breed, which is very resistant to heat due to its larger body surface area and greater number of sweat glands. The characteristics of its hair also facilitate heat exchange with the environment. In addition, the digestive tract is 10% smaller than that of European cattle breeds (ACNB, 2022). Therefore, their metabolism is lower and generates less heat. According to Dewell (2010), larger cattle cannot handle heat stress as well as smaller cattle because increased fat deposition prevents cattle from regulating their heat effectively. Solar radiation is a critical component that can lead to losses from heat stress. In our study, the average weight was 300 kg which contributed to these animals being able to handle the heat. This result agrees with values for ultimate pH obtained by Lacerda et al. (2022) who investigated the effects of different pre-slaughter road transport times on sensory evaluation and instrumental measurements of meat quality of Nellore cattle. They observed that, with the longest transport being 6 h, no noticeable effect on ultimate pH or meat quality.

Weak but significant correlations were obtained between ultimate pH and carcass weight ($r = 0.12$, $P < 0.01$) on the one hand and, between distance and carcass weight on the other ($r = 0.05$, $P < 0.01$). This is in agreement with González et al. (2012a), which aimed to identify and quantify several factors affecting shrinkage in cattle during long-haul commercial transport (≥400 km; $n = 6{,}152$ trips). They concluded that transport duration was the variable with the most influence on shrinkage, especially at high ambient temperatures because both factors have a multiplicative effect on each other. Thus, every attempt should be made to reduce transport duration and shrinkage, such as by avoiding unnecessary delays through careful trip planning and efficient border crossing inspection protocols for feeder cattle. Transport should be more carefully managed during hot weather to minimize avoidable shrinkage. Again, hot carcass weight was found not to be affected by pre-slaughter road transport distance. This may be because even the longest transport time added to the waiting time in the holding pen was insufficient to reduce carcass weight. In addition, other factors related to transportation

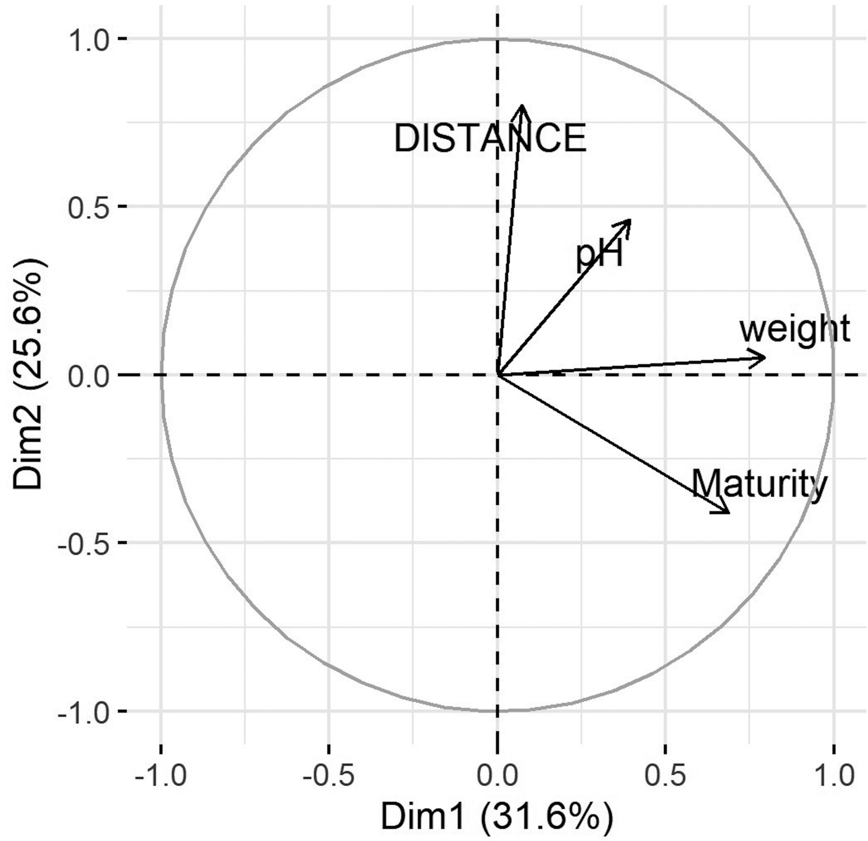

Figure 5. Principal component analysis. This analysis was performed using the following variables: distance, ultimate pH, weight, maturity. Projection of variables in a "XY" plane defined by the axes for the first two principal components (PC1 and PC2), showing the percentage of explained variability in the two PCs of the plot.

can contribute to changes in carcass weight, such as animal density, and lot mixing in truck compartments (Mendonça et al., 2019). However, in the current study, these factors were controlled for, so that the effects of transport distance could be isolated and reliably determined (Table 3).

Transportation distance and maturity had a negative weak relationship (r = –0.03, P < 0.01), with a low correlation between carcass weight and maturity (r = 0.23, P < 0.01). This significant correlation is due to the fact that the age at which an animal is slaughtered determines its weight and composition of the carcass because of its stage of maturity (Pethick et al., 2007).

In the PCA, the distance between farm and slaughterhouse, carcass weight, maturity, and ultimate pH were considered simultaneously in the right upper third of the plot. This shows why the first two PCAs explain only 57.2% of the total variability of the dataset, with 31.6% corresponding to the first principal component and 25.6% to the second principal component. Results from Principal Component Analysis (PCA) confirmed a low correlation between ultimate pH and distance and a significant correlation between maturity and carcass weight (Figure 5). The contribution of the ultimate pH in the first dimension is 16.82% and in the second dimension is 11.91%; distance from farm to slaughterhouse contributes 2.16% and 63.25%, in the first and second dimensions, respectively; carcass weight contributes 49.47% in the first dimension and 0.09% in the second dimension; and animal maturity contributes 31.55% and 24.76% in the first and second dimensions, respectively.

However, these results should be interpreted with caution due to the large amount of noise in the data. Because of this, it is important to emphasize that in the current study, our protocol was insufficient to adequately test our hypothesis, because the primary objective of the present case study was to make an exploratory analysis of the company's data, taking into consideration all the data obtained during the 6-mo period evaluated, to have a robust dataset with pre- and post-slaughter data.

pH as a Function of Carcass Weight, Maturity, Distance

Results from the regression analysis showed that distance does not have a significant effect on pH (P = 0.634). Carcass weight significantly affects pH (P < 0.001) with a low coefficient of 0.000266, and, as such, has a very small effect.

Animal maturity (age) negatively affects pH as well (P < 0.001) but also with a very small effect (the coefficient being –0.000806).

$$y_i = 5.67 + 0.000266x_{i1} - 0.000806x_{i2} + 0.00000165x_{i3}$$

Where y_i represents the measured ultimate pH, x_{i1} represents the carcass weight, x_{i2} represents the animal maturity evaluated for the carcass, x_{i3} represents the distance between the farm and the slaughterhouse.

The value of R^2 was 0.016, which indicates that only 1.6% of total variability can be explained by this regression model. Therefore, 98.4% of the variability is attributable to other

Table 4. Point estimate of variance components of random effects and model error respectively, using the restricted maximum likelihood method. Lower and upper bounds of 95% confidence intervals of the variance components of random effects and model error

Variances	Point estimate	Lower limit	Upper limit
Random effects	0.02979055	0.02698031	0.0328935
Random error	0.06518885	0.06466902	0.06571286

factors not considered here. Thus, results from the multiple regressions model confirm the previous results from the PCA. However, the effects even though small were detectable due to the large sample size.

pH Variability Between and Within Farms

Results from 95% confidence intervals of variance of the random effects and of the random error showed that the variability within farms is higher than between farms (Table 4).

These results may be explained by the fact each farm is at a different distance from the slaughterhouse, and by each farm having provided several carcasses with different characteristics. Because of this, the variability of the pH of the carcasses provided by each farm is greater than the variability of carcass pH between farms. The estimate of the random effect variance is smaller than the estimate of the random error variance of the model. This may be due to the high variability in the characteristics of carcasses and in meat quality of cattle is multifactorial. Indeed, the high variability as reported by Clinquart et al. (2022) is often linked to the priorities of the farming system. The conclusion reached was that the diversity of farming systems is a consequence of the diversity in several factors: breed (dairy or beef), age, and sex (bulls, steers, heifers, cull cows) used to produce beef. Further, there are other factors linked to farming practices (e.g., diet, especially grazing) that have a strong influence on the sensory, nutritional, technological, and extrinsic attributes of meat quality (Clinquart et al., 2022).

Liu et al. (2022) also reported the synergies and antagonisms between beef quality dimensions. To give two examples: the effect of genetics alone (highly muscled breeds, local breeds, etc.), and different diets (pasture *vs* inland, organic *vs* conventional on grass) have different effects on the attributes of meat quality. These two factors vary to a greater or lesser degree within a given farm's herd as well as between the herds of different farms. Although the study of genetic improvement of the Nellore breed is widespread in Brazil, the animals used in the present study were not genetically identified as Nellore either individually or in aggregate but were classified as Nellore by the farms. This results in variability of carcass characteristics, even when they come from the same farm as reported in the literature, even when the animals are of the same gender. This justifies the result obtained through the mixed model (Table 4).

It is worth noting that improvements at the farm level can be impeded or even eliminated by poor transport, substandard slaughter and processing practices. All these considerations explain why inter-animal variability in quality can be high, even when a population of animals are products of the same farming system (Duarte et al., 2011). In the present study, pre- and post-slaughter protocols were those recommended by Brazilian legislation.

Modeling the Relationship Between Carcass Weight and Maturity and Farms

Results from a linear mixed model show that maturity has a significant effect on the carcass weight ($P < 0.001$) with a large coefficient of 2.90. A ratio likelihood test outperformed the mixed model multiple regression. The value of R^2 in the linear mixed model is 46.03%, which indicates that this total variability can be explained by the mixed regression model. Therefore, 53.97% of the variability is attributable to other factors not considered here. The Linear Mixed Model of the relationship between carcass weight and maturity and the farms confirms the results for Pearson's correlation between carcass weight and maturity (Table 3), as well as by PCA (Figure 5). As previously noted, these results were also found by other researchers (Bureš and Bartoň, 2012; Silva et al., 2019). Consistent with Pethick et al. (2007), Silva et al. (2019), and Bureš and Bartoň (2012), we conclude that the age at which an animal is slaughtered determines the weight and composition of the carcass based on the stage of maturity reached.

Several factors should be considered when developing guidelines to reduce cattle transport stress and shrinkage: the type of cattle, ambient temperature, transport duration, driving quality, and time and origin of loading (González et al., 2012a).

Conclusion

Beef carcass traits can be affected by carcass weight and maturity, but only to a limited extent. The hypothesis that long distances would significantly affect beef quality was not confirmed for the Nellore breed in the conditions studied. This could be because of low stress during transport, as well as the physical characteristics of the Nellore breed that favor greater resistance to tropical climatic conditions.

In addition, the strategy of purchasing animals within a radius of 300 km, equivalent to a maximum of 8 h of transport time, is consistent with the literature on changes in the quality of bovine carcasses due to transport time. This is an observation beneficial to the company and conforms to the standards required by the Brazilian market and to those markets, to which Brazil exports.

An experimental study on a much smaller scale, with an assessment of stress indicators, such as lactate, glucose concentrations, cortisol concentration and creatine kinase, norepinephrine and epinephrine, or bruise score sheet to evaluate the consequences on beef eating quality, is necessary to understand any interaction between these parameters and the benefits for the supply chain so that the meat industry can further improve animal welfare and meat quality during pre-slaughter commercial operations. This study which used a dataset provided by a slaughterhouse can serve as a source of useful indicators on a large scale. Although meat production has become more efficient and line speed has increased, there is still room for improvement throughout the pre-slaughter logistics chain.

Acknowledgments

NM's scholarship was sponsored by FAPEG/CAPES (Fundação de Amparo à Pesquisa do Estado de Goiás / Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), the French Embassy in Brazil and the Beauvallet company. This research was partly supported by the INTAQT project that has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°101000250.

Conflict of interest statement. None of the authors have any conflicts of interest to declare.

Literature Cited

ABIEC—Associação Brasileira das Indústrias Exportadoras de Carne. 2022. Beef REPORT Perfil da Pecuária no Brasil 2022, São Paulo, Brasil

ACNB—Associação dos Criadores de Nelore do Brasil. 2022. nelore.org.br

Apaoblaza, A., S. D. Gerrard, S. K. Matarneh, J. C. Wicks, L. Kirkpatrick, E. M. England, T. L. Scheffler, S. K. Duckett, H. Shi, S. L. Silva, et al. 2020. Muscle from grass and grain-fed cattle differs energetically. Meat Sci. 161:107996. doi:10.1016/j.meatsci.2019.107996

Behrendt, S. 2014. Lm. Beta: add standardized regression coefficients Lm-objects. R package version 1. 5–1

Brasil. 2004. Ministério da Agricultura, Pecuária e Abastecimento. Mapa. Instrução Normativa Mapa nº 09, de 04 de maio de 2004, sobre Sistema Brasileiro de Classificação de Carcaças de Bovinos, em todo o território nacional, e a classificação dos bovinos abatidos nos estabelecimentos sob o controle do Serviço de Inspeção Federal (SIF), conforme consta do Anexo desta Instrução Normativa

Brasil. 2021a. Ministério da Agricultura, Pecuária e Abastecimento. Mapa. Instrução Normativa Mapa nº 365, de 16 de julho de 2021, aprova o Regulamento Técnico de Manejo Pré-abate e Abate Humanitário e os métodos de insensibilização autorizados pelo Ministério da Agricultura, Pecuária e Abastecimento

Brasil. 2021b. Ministério da Agricultura, Pecuária e Abastecimento. Mapa. Instrução Normativa Mapa nº 09, de 16 de junho de 2021, aprova o modelo impresso da Guia de Trânsito Animal (GTA) para o trânsito de animais vivos, ovos férteis e outros materiais de multiplicação animal e estabelece o formato eletrônico da Guia de Trânsito Animal (GTA), na forma do modelo e-GTA, para movimentação, em todo o território nacional, de animais vivos, ovos férteis e outros materiais de multiplicação animal

Brasil. 2022. Instituto Nacional De Meteorologia Do Brasil – INMET. Normais Climatológicas. Brasília - DF. https://portal.inmet.gov.br/

Bureš, D., and L. Bartoň. 2012. Growth performance, carcass traits and meat quality of bulls and heifers slaughtered at different ages. Czech J. Anim. Sci. 57:34–43. doi:10.17221/5482-CJAS.

Chulayo, A. Y., G. Bradley, and V. Muchenje. 2016. Effects of transport distance, lairage time and stunning efficiency on cortisol, glucose, HSPA1A and how they relate with meat quality in cattle. Meat Sci. 117:89–96. doi:10.1016/j.meatsci.2016.03.001

CIBALLAHAL—Central IslâmicaBrasileira De Alimentos Halal. 2022. https://www.fambrashalal.com.br/empresas-certificadas. [Accessed on 22 September 2022]

Clinquart, A., M. P. Ellies-Oury, J. F. Hocquette, L. Guillier, V. Santé-Lhoutellier, and S. Prache. 2022. Review: on-farm and processing factors affecting bovine carcass and meat quality. Animal. 16:100426. doi:10.1016/j.animal.2021.100426

Cooke, R. F., C. L. Daigle, P. Moriel, S. B. Smith, L. O. Tedeschi, and J. M. B. Vendramini. 2020. Cattle adapted to tropical and subtropical environments: social, nutritional, and carcass quality considerations. J. Anim. Sci. 98:1–20. doi:10.1093/jas/skaa014

De Andrade, T. S., T. Z. Albertini, L. G. Barioni, S. R. De Medeiros, D. D. Millen, A. C. R. Dos Santos, R. S. Goulart, and D. P. D. Lanna. 2020. Perception of consultants, feedlot owners, and packers regarding the optimal economic slaughter endpoint in feedlots: a national survey in Brazil (Part I). Can. J. Anim. Sci. 100:745–758. doi:10.1139/cjas-2019-0219

Deng, L., C. He, Y. Zhou, L. Xu, and H. Xiong. 2017. Ground transport stress affects bacteria in the rumen of beef cattle: a real-time PCR analysis. Anim. Sci. J. 88:70–797. doi:10.1111/asj.12615

Devlin, D. J., N.F.S. Gault, B.W. Moss, E. Tolland, J. Tollerton, L.J. Farmer, and A. W. Gordon. 2017. Factors affecting eating quality of beef. Adv. Anim. Biosci. 8:s2–s5. doi:10.1017/S2040470017001583

Dewell G. 2010: Heat Stress in Beef Cattle. Iowa State University, Ames. [Accessed 27 September 2023] https://vetmed.iastate.edu/vdpam/about/production-animal-medicine/beef/bovine-disease-topics/heat-stress-beef-cattle

Duarte, M. S., P. V. R. Paulino, M. A. Fonseca, L. L. Diniz, J. Cavali, N. V. L. Serao, L. A. M. Gomide, S. F. Reis, and R. B. Cox. 2011. Influence of dental carcass maturity on carcass traits and meat quality of Nellore bulls. Meat Sci. 88:441–446. doi:10.1016/j.meatsci.2011.01.024

DuToit, S. H., A. G. W. Steyn, and R. H. Stumpf. 2012. Graphical exploratory data analysis. Springer Science & Business Media

Ferreira, G. B., C. L. Andrade, F. Costa, M. Q. Freitas, T. J. P. Silva, and I. F. Santos. 2006. Effects of transport time and rest period on the quality of electrically stimulated male cattle carcasses. Meat Sci. 74:459–466. doi:10.1016/j.meatsci.2006.04.006

González, L. A., K. S. Schwartzkopf-Genswein, M. Bryan, R. Silasi, and F. Brown. 2012b. Benchmarking study of industry practices during commercial long haul transport of cattle in Alberta, Canada. J. Anim. Sci. 90:3606–3617. doi:10.2527/jas.2011-4770

González, L. A., K. S. G. Schwartzkopf-Genswein, M. Bryan, R. Silasi, and F. A. Brown. 2012a. Factors affecting body weight loss during commercial long haul transport of cattle in North America. J. Anim. Sci. 90:3630–3639. doi:10.2527/jas.2011-4786

Gruber, S. L., J. D. Tatum, T. E. Engle, P. L. Chapman, K. E. Belk, and G. C. Smith. 2010. Relationships of behavioral and physiological symptoms of pre slaughter stress to beef longissimus muscle tenderness. J. Anim. Sci. 88:1148–1159. doi:10.2527/jas.2009-2183

Hemsworth, P. H., M. Rice, M. G. Karlen, L. Calleja, J. L. Barnett, J. Nash, and G. J. Coleman. 2011. Human-animal interactions at abattoirs: relationships between handling and animal stress in sheep and cattle. Appl. Anim. Behav. Sci. 135:24–33. doi:10.1016/j.applanim.2011.09.007

Hughes, J., F. Clarke, P. Purslow, and R. Warner. 2017. High pH in beef longissimus thoracis reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark colour. Food Res. Int. 101:228–238. doi:10.1016/j.foodres.2017.09.003

Husson, F., Josse, J., Le, S., Mazet, J. 2016. Multivariate exploratory data analysis and data mining. R Package

Lacerda, N. G., R. Mezzomo, I. M. de Oliveira, K. S. Alves, L. R. S. de Oliveira, M. C. A. Santos, R. R. F. Lima, and D. I. Gomes. 2022. Pre-slaughter road transportation times: meat quality and sensory properties of Nellore bull meat. Can. J. Anim. Sci. 102:30. doi:10.1139/cjas-2020-0032

Liu, J., M. P. Ellies-Oury, T. Stoyanchev, and J. F. Hocquette. 2022. Consumer perception of beef quality and how to control, improve and predict it? Focus on eating quality. Foods. 11:1732. doi:10.3390/foods11121732

Lomiwes, D., M. M. Farouk, G. Wu, and O. A. Young. 2014. The development of meat tenderness is likely to be compartmentalized by ultimate pH. Meat Sci. 96:646–651. doi:10.1016/j.meatsci.2013.08.022

Maria, G. A., M.Villarroel, , C. Sanudo, J. L. Olleta, and G. Gebresenbet. 2003. Effect of transport time and aging on aspects of beef quality. Meat Sci. 65:1335–1340. doi:10.1016/S0309-1740(03)00054-8

Mello, J. L. M., A. B. B. Rodrigues, A. Giampietro-Ganeco, F. B. Ferrari, R. A. Souza, P. A. Souza, and H. Borba. 2018. Characteristics of carcasses and meat from feedlot-finished buffalo and *Bos indicus* (Nellore) bulls. Anim. Prod. Sci. 58:1366–1374. doi:10.1071/an16556

Mendonça, F. S., R. Z. Vaz, F. F. Cardoso, J. Restle, F. N. Vaz, L. L. Pascoal, and A. A. Boligon. 2018. Pre-slaughtering factors related to bruises on cattle carcasses. Anim. Prod. Sci. 58:385–392. doi:10.1071/AN16177

Mendonça, F. S., R. Z. Vaz, F. N. Vaz, W. S. Leal, I. D. B. Silveira, J. Restle, A. A. Boligon, and F. F. Cardoso. 2019. Causes of bruising in carcasses of beef cattle during farm, transport, and slaughterhouse handling in Brazil. Anim. Sci. J. 90:288–296. doi:10.1111/asj.13151

Nielsen, B. B., L. Dybkjaer, and M. S. Herskin. 2011. Road transport of farm animals: effects of journey duration on animal welfare. Animal. 5:415–427. doi:10.1017/S1751731110001989

Pérez Linares, C., E. Sánchez López, F. Ríos Rincón, J. Olivas Valdés, F. Fegueroa Saavedra, and A. Barreras Serrano. 2013. Pre and post slaughter cattle and carcass management factors associated to presence of DFD beef in the hot season. Rev. Mex. Cienc. Pecu. 4:149–160

Pethick, D.W., Barendse, W., Hocquette, J.F., Thompson, J., Wang, Y.H. 2007. Regulation of marbling and body composition—growth and development, gene markers and nutritional biochemistry. In: Proceedings of the 2nd International Symposium on Energy and Protein Metabolism and Nutrition, 9–13 September 2007, Vichy, France; pp. 75–88

Pinheiro J, Bates D, R Core Team. 2022. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–157. https://CRAN.R-project.org/package=nlme

Pinheiro, J. C., and D. M. Bates. 2000. Linear mixed-effects models: basic concepts and examples. Mixed-effects models in S and S-Plus; Springer: New York, 3–56

Pogorzelski, G., E. Pogorzelska-Nowicka, P. Pogorzelski, A. Półtorak, J. -F. Hocquette, and A. Wierzbicka. 2022. Towards an integration of pre- and post-slaughter factors affecting the eating quality of beef. Livest. Sci. 255:104795. doi:10.1016/j.livsci.2021.104795

Polkinghorne, R., J. Philpott, and J. M. Thompson. 2018. Do extended transport times and rest periods impact on eating quality of beef carcasses? Meat Sci. 140:101–111. doi:10.1016/j.meatsci.2018.02.017

R Core Team. 2022. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria. [Accessed May 15, 2022] http://www.R-project.org/

Schwartzkopf-Genswein, K. S., L. Faucitano, S. Dadgar, P. Shand, L. A. González, and T. G. Crowe. 2012. Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: a review. Meat Sci. 92:227–243. doi:10.1016/j.meatsci.2012.04.010

Seideman, S. C., H. R. Cross, R. R. Oltjen, and B. D. Schanbacher. 1982. Utilization of the intact male for red meat production: a review. J. Anim. Sci. 55:826–840. doi:10.2527/jas1982.554826x

Silva, L. H. P., D. E. F. Assis, M. M. Estrada, G. J. F. Assis, G. D. R. Zamudio, G. B. Carneiro, S. C. Valadares Filho, M. F. Paulino, and M. L. Chizzotti. 2019. Carcass and meat quality traits of Nellore young bulls and steers throughout fattening. Livest. Sci. 229:28–36. doi:10.1016/j.livsci.2019.09.012

Strappini, A. C., J. H. M. Metz, C. B. Gallo, and B. Kemp. 2009. Origin and assessment of bruises in beef cattle at slaughter. Animal. 3:728–736. doi:10.1017/S1751731109004091

Warren, L. A., I. B. Mandell, and K. G. Bateman. 2010. Road transport conditions of slaughter cattle: Effects of prevalence of dark, firm and dry beef. Can. J. Anim. Sci. 90:471–482. doi:10.4141/cjas09091

Werner, M., C. Hepp, C. Soto, P. Gallardo, H. Bustamante, and C. Gallo. 2013. Effects of a long distance transport and subsequent recovery in recently weaned crossbred beef calves in Southern Chile. Livest. Sci. 152:42–46. doi:10.1016/j.livsci.2012.12.007