

# Physical and microbiological quality and oxidative stability in different storage periods of Angus-Nellore heifer meat in an integrated livestock-forest system

## Qualidade física e microbiológica e estabilidade oxidativa em diferentes períodos de armazenamento da carne de novilhas Angus-Nelore em integração pecuária-floresta

Patrícia Aparecida da Luz Zanetti<sup>1\*</sup>; Gustavo Pavan Mateus<sup>2</sup>; Gelci Carlos Lupatini<sup>3</sup>; Leonardo Henrique Zanetti<sup>1</sup>; Natália Carolina Vieira<sup>4</sup>; Franciely de Oliveira Neves<sup>5</sup>; Juliana Mara de Freitas Santos<sup>5</sup>; Gustavo Antunes Trivelin<sup>5</sup>; Estevão Moretti Mazetti<sup>6</sup>; Cristiana Andrighetto<sup>3</sup>

### Highlights

Integrated systems allow production in a sustainable way and promote animal welfare.  
Trees do not alter the physical, microbiological and oxidative quality of the meat.  
Storage time increases meat lipid oxidation and bacteria count.

### Abstract

This study aimed to evaluate the physical and microbiological quality as well as the oxidative stability of meat stored frozen (30, 60, 90, 180, and 360 days) and obtained from Angus-Nellore heifers kept in a conventional system (CS; no shade available) and Integrated Livestock-Forest (ILF-1L and ILF-3L). Forty-eight ½ Nellore ½ Angus heifers with an average initial weight of approximately  $276.70 \pm 20.1$  kg and an average age of nine months were distributed across a randomized block design with three treatments

<sup>1</sup> Profs. Drs., Department of Agricultural Sciences, Universidade do Oeste Paulista, UNOESTE, Presidente Prudente, SP, Brasil. E-mail: patriciazanetti@unoeste.br; leonardozanetti@unoeste.br

<sup>2</sup> Researcher, Agência Paulista de Tecnologia dos Agronegócios, Polo Regional Extremo Oeste, Andradina, SP, Brasil. E-mail: gpmateus@apta.sp.gov.br

<sup>3</sup> Profs. Drs., Departamento de Produção Animal, Faculdade de Ciências Agrárias e Tecnológicas, Universidade Estadual Paulista, UNESP, Dracena, SP, Brasil. E-mail: gelci.lupatini@unesp.br; cristiana.andrighetto@unesp.br

<sup>4</sup> Student of the Doctoral Course of the Postgraduate Program in Animal Science, UNOESTE, Presidente Prudente, SP, Brasil. E-mail: natalia.carolina@hotmail.com

<sup>5</sup> Students of the Master's Course of the Postgraduate Program in Animal Science, Faculdade de Ciências Agrárias e Tecnológicas, UNESP, Dracena, SP, Brasil. E-mail: franciely.neves@hotmail.com; julianazootec@yahoo.com.br; gustavotrivelin@hotmail.com

<sup>6</sup> Graduated in Animal Science, Faculdade de Ciências Agrárias e Tecnológicas, UNESP, Dracena, SP, Brasil. E-mail: estevaommazetti@gmail.com

\* Author for correspondence

and four replicates. After slaughter, the Longissimus thoracis muscle was collected from each carcass for pH, lipid oxidation, meat color, and microbiological analyses. The physical and microbiological quality and oxidative stability of the meat were not affected by the tree component present in the pasture. Regarding the duration of frozen storage, it was found that the pH, meat color ( $a^*$ ,  $b^*$ ,  $C^*$ ,  $H^*$ ,  $O/M$ ), and lipid oxidation increased as the period of storage increased ( $P < 0.05$ ). The physical and microbiological quality, as well as the oxidative stability of the meat, was not affected by the tree component present in the pasture. However, the meat changed as the frozen storage time increased.

**Key words:** Industrial crossing. Longissimus thoracis. pH. Silvopastoral system. Total bacteria count.

## Resumo

O objetivo deste estudo foi avaliar a qualidade física e microbiológica e a estabilidade oxidativa da carne congelada (30, 60, 90, 180 e 360 dias) de novilhas Angus-Nelore em sistema convencional (CS; sem sombra disponível) e Integração Pecuária-Floresta (ILF-1L e ILF-3L). Quarenta e oito novilhas  $\frac{1}{2}$  Nelore  $\frac{1}{2}$  Angus com peso médio inicial de aproximadamente  $276,70 \pm 20,1$  kg e idade média de nove meses foram distribuídas em delineamento em blocos casualizados com três tratamentos e quatro repetições. Após o abate, o músculo Longissimus thoracis foi coletado de cada carcaça para análise de pH, oxidação lipídica, cor da carne e análise microbiológica. A qualidade física, microbiológica e a estabilidade oxidativa da carne não foram afetadas pelo componente arbóreo presente na pastagem. Em relação aos dias de armazenamento sob congelamento, verificou-se que o pH da carne  $a^*$ ,  $b^*$ ,  $C^*$ ,  $H^*$ ,  $O/M$  e a oxidação lipídica aumentaram com o tempo de armazenamento da carne. A qualidade física e microbiológica e a estabilidade oxidativa da carne não são afetadas pelo componente arbóreo da pastagem. No entanto, a carne muda à medida que o tempo de armazenamento sob congelamento aumenta.

**Palavras-chave:** Contagem total de bactérias. Cruzamento industrial. *Longissimus thoracis*. pH. Sistema silvipastoril.

## Introduction

Integrated systems, such as crop-livestock integration and crop-livestock-forest integration (CLF), are emerging as a strategy for the expansion of global food production due to their conservationist and sustainable nature. Moreover, such systems are gaining prominence on the national scene because they reduce the financial and productive risk in the agricultural sector and cause less natural destruction compared with traditional agricultural practices

(Barbosa et al., 2015). With the inclusion of trees, new advantages have been added to the system, including the availability of wood on the property for different uses, an increase in the income per unit of area, and multiple benefits for a large number of rural establishments, with a consequent increase in the social conditions of the people who live in these areas, often in situations of poverty, and have agriculture and livestock breeding as their only productive activity (Food and Agriculture Organization of the United Nations [FAO], 2016).

Among the advantages of CLF systems, the presence of trees in the pasture can also benefit the animals that live there, protecting them from adverse weather conditions and favoring their welfare, with a consequent increase in meat production (Domiciano et al., 2016). However, current works cited in the literature (Oliveira et al., 2022; Portugal et al., 2023) evaluate the quantity of products from agriculture and livestock, such as forage mass and animal weight gain, whereas meat quality has rarely been evaluated in these systems.

In a review of how climate change can affect meat quality, Gregory (2010) reports that the effects on metabolism are directly related to the decrease in muscle pH during slaughter. This occurs as a result of the accumulation of lactic acid produced from glycogen during anaerobic glycolysis (Forrest et al., 1979). Thus, when an animal undergoes heat stress for long periods, the animal's muscle glycogen may be almost completely depleted and may not produce sufficient lactic acid to decrease the pH ( $\text{pH} > 6.0$ ) (Gomide et al., 2014), giving the meat a dark color and a firm, dry texture (Melo et al., 2016). However, there are not enough data showing that with the higher pH, there is also a greater development of deteriorating microorganisms in meat (Alcantara et al., 2012).

Studies report that in some parts of the world, during the summer, when temperatures are higher, there is an increase in the number of cases of diarrhea or food poisoning in the population, which may be linked to contaminated meat, explained by an increase in postmortem pH, which occurs in exhausted animals or those who

have suffered heat stress for a long period. This makes beef an ideal culture medium for numerous microorganisms (Domínguez et al., 2007). In addition to microbiological quality, lipid oxidation can also be affected by heat stress (Lu et al., 2017; Janni et al., 2020). In general, high temperatures affect the animal's body, increasing the production of free radicals, which, when not balanced by the antioxidant protection system, is called oxidative stress (Van der Pol et al., 2019), observed as an increase in lipid oxidation.

The changes in pH and lipid oxidation occurring in meat as a result of heat stress leave much to discover regarding the changes that can occur in this product during long periods of storage. Thus, studies that include the effects of the freezing technique on meat in sustainable systems, as well as the possible changes in its quality in the long term are also essential, since freezing is a widely used meat preservation method, above all, in the beef trade between countries, where the distance between producer and consumer dictate the use of cooling or freezing as a tool to ensure the quality of meat during transport and storage.

Thus, there is a need to improve knowledge about silvopastoral systems, aiming to reconcile the increased demand for adequate food in the hygienic-sanitary aspect with the urgent need for sustainable agriculture. The objective of this study was to evaluate the physical and microbiological quality, as well as the oxidative stability during different periods of frozen storage, in meat obtained from crossbred heifers kept in conventional and silvopastoral systems with two tree densities.

## Material and Methods

The experiment was carried out in accordance with the ethical principles for animal tests (Protocol N° 03/2017. R1) determined by the Ethics Committee on Animal Use (CEUA) of the College of Agricultural and Technological Sciences, São Paulo State University (Unesp), Brazil.

### *The experimental site and the climatic conditions*

The experiment was conducted at the Paulista Agency for Agribusiness Technology (APTA), Far West Regional Pole, located in the municipality of Andradina (20°53'38" south latitude, 51°23'1" west longitude at an altitude of 400 m), west of the São Paulo state.

According to the Köppen climate classification system, the predominant climate in the region is Aw, characterized as highland tropical, with a hot and rainy summer and a dry winter (Alvares et al., 2013). As shown by the agency's weather data, the region's annual precipitation is around 1,257 mm, with 78% of the rainfall occurring in October-April and 22% in May-September, which, consequently, corresponds to the dry season. Historically (1956-2013), the average annual maximum and minimum temperatures were 30.7°C and 17.1°C, respectively, with an average annual precipitation of 1,181.6 mm (Universidade Estadual de Campinas [UNICAMP], 2012). Climatic and thermal comfort data were measured during the experimental period (Table 1).

**Table 1**

**Descriptive analysis of climatic conditions and thermal comfort in the conventional systems (CS) and Integrated Livestock-Forest (ILF) with a density of 196 trees ha<sup>-1</sup> (ILF-1L) and 448 trees ha<sup>-1</sup> (ILF-3L), Andradina - Sao Paulo**

Climatic characteristics <sup>1</sup>	Treatments - 2017*				
	CS	ILF-1L	ILF-3L	Max.	Min.
Wind speed (m/s)	1.42 ± 1.23	1.06 ± 1.32	0.95 ± 1.16	3.24	0.01
Dry Bulb Temp (°C)	26.58 ± 8.92	25.51 ± 7.75	25.48 ± 7.98	34.94	16.86
Globe Temp (°C)	33.23 ± 9.70	28.05 ± 8.50	29.20 ± 9.13	41.74	18.06
Relative humidity (%)	54.95 ± 1.86	57.05 ± 2.22	56.72 ± 2.71	60.84	52.17
Radiant Thermal Load (W.m <sup>-2</sup> )	564.46 ± 48.80	500.57 ± 50.46	510.36 ± 45.59	621.87	427.80
Temp and Humidity Index	73.96 ± 11.87	72.83 ± 10.49	72.86 ± 10.68	85.10	61.37
Globe Temp and Humidity Index	80.93 ± 12.98	74.98 ± 11.58	76.17 ± 12.21	91.84	62.38
Climatic characteristics <sup>1</sup>	Treatments - 2018*				
	CS	ILF-1L	ILF-3L	Max.	Min.
Wind speed (m/s)	1.39 ± 1.42	1.02 ± 1.06	1.00 ± 0.95	5.70	0.00
Dry Bulb Temp (°C)	26.58 ± 5.04	25.01 ± 4.26	24.78 ± 4.09	34.25	14.34
Globe Temp (°C)	31.09 ± 7.21	27.45 ± 5.89	27.12 ± 5.21	47.81	14.46
Relative humidity (%)	54.97 ± 18.80	58.06 ± 17.56	58.56 ± 17.28	95.59	32.66
Radiant Thermal Load (W.m <sup>-2</sup> )	486.55 ± 43.83	464.33 ± 35.29	462.15 ± 31.14	551.77	387.99
Temp and Humidity Index	73.96 ± 4.94	74.56 ± 4.17	74.17 ± 3.92	81.44	60.57
Globe Temp and Humidity Index	77.78 ± 6.98	74.56 ± 5.78	74.17 ± 5.02	87.94	60.69

\*Experimental period: June 2017 to July 2018.

<sup>1</sup>Data collected in the experimental area.

### Area history and experimental period

The experiment was conceived in the first half of 2012, when specific treatments were selected and the division of pickets was carried out. The soil of the experimental area was classified as Dystrophic Yellow Red Latosol (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2013) with sandy surface layer and mean terrain slope of 6.0%. In July 2012, the type of the area's soil was corrected based on the results of the chemical analyzes (0-20 cm), which revealed

the following attributes: pH (CaCl<sub>2</sub>) 4.8; M.O. 16 g dm<sup>-3</sup>; P (resin) 3 mg dm<sup>-3</sup>; K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and H+Al 1.9; 7.0; 5.0 and 20 mmolc dm<sup>-3</sup>, respectively, S-SO<sub>4</sub><sup>2-</sup> 1.0 mg dm<sup>-3</sup> and V% (base saturation) of 42%. The clay, silt and sand contents were 107; 113 and 780 g kg<sup>-1</sup>, respectively. Dolomitic limestone (PRNT 80%) and gypsum were applied and incorporated into the soil, as recommended by Bulletin 100 (Van Raij et al., 1997) for the São Paulo state. When preparing the soil, terracing, harrowing, plowing and leveling were performed.

The trees were introduced from November 2012 to March 2013 through manual planting of the seedlings, following the level curves present in the area (Porfírio-da-Silva et al., 2010). The eucalyptus clone used in the planting was the I-224 of *Eucalyptus urograndis*, oriented towards cellulose production, which is the commercial characteristic of the plantation region. The seedlings were fertilized using 350 kg ha<sup>-1</sup> of the 04-30-16 formula, with 210 g of fertilizer used per seedling (8.4 g N, 63 g P<sub>2</sub>O<sub>5</sub>, 33.6 g of K<sub>2</sub>O) in each planting pit. During the top dressing phase carried out in February 2013, 37 kg ha<sup>-1</sup> of nitrogen, 3 kg ha<sup>-1</sup> of zinc and 2 kg ha<sup>-1</sup> of boron were used, applying 50 g of urea (23 g N), 9 g of zinc sulfate (1.8 g Zn) and 12 g of borogran (1.2 g B) in the form of a crown around each eucalyptus seedling. In January 2014, another top dressing round was carried out with 123 kg ha<sup>-1</sup> of N and using 160 g of urea (73.6 g N) in the form of a crown around each seedling.

The average height of the eucalyptus during the experimental period was 18.0±4.7 and 18.2±4.6 m, while the diameter at breast height was 17.8±4.3 and 17.6±4.0 cm for ILF-1L and ILF-3L, respectively. The eucalyptus trees occupied 8% and 28% of the paddock area in ILF-1L in ILF-3L, respectively.

The seeding of soybeans (cultivar BMX Power) was performed in December 2012 in all systems (ILP, ILPF-1L and ILPF-3L), totaling 400,000 seeds per ha<sup>-1</sup>. The mineral fertilization of seedlings corresponded to the application of 12 kg ha<sup>-1</sup> of N, 90 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 48 kg ha<sup>-1</sup> of K<sub>2</sub>O. The top dressing was carried out 40 days after planting, applying 200 kg ha<sup>-1</sup> of the 00-20-20 formula. The post-emergent control of weeds was carried out on 01/24/2013, applying herbicide based

on Glyphosate (Zapp QI 620) at a dose of 1,240 g i.a. ha<sup>-1</sup>. During this application, cobalt and molybdenum-based fertilizer (COMO Platinum) was mixed in a tank and used in the proportion of 150 mL ha<sup>-1</sup> of the commercial product. The soybean harvest was carried out in May 2013, yielding an average of 35 bags ha<sup>-1</sup>. After the soybean harvest, weed control was performed in the area. The area was desiccated using Glyphosate-based herbicide (Roundup WG) at a dose of 1440 g a.i. ha<sup>-1</sup>, with a total applied volume of 250 L ha<sup>-1</sup>.

In December 2013 the grass was sown, using Marandu cultivar of *Urochloa brizantha* (Syn. *Brachiaria brizantha*) in the amount of 8.0 kg ha<sup>-1</sup> of pure and viable seeds, planted with spacing between rows of 0.20 m. In planting the maize, a 0.80 m spacing was used between lines, aiming to reach a population density of 62,500 plants per hectare, while the fertilization of seedlings corresponded to 24.8 kg ha<sup>-1</sup> of N, 86.8 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 49.6 kg ha<sup>-1</sup> of K<sub>2</sub>O. 20 days after the emergence of maize plants, top dressing was performed using 92 kg ha<sup>-1</sup> of nitrogen.

The maize was harvested in April 2014, and fences were built in this period. Then the drinking fountains were installed and the area remained untouched until the entrance of the animals. Between December 08, 2014 and January 9, 2015, the forage was standardized by means of mechanical weeding at a height of 15 cm, followed by nitrogen fertilization, applying 40 kg ha<sup>-1</sup> of N in the form of urea. The first experiment in the experimental area was carried out from January 2015 to July 2016 and involved Nellore cattle. The present study is the second cycle of the system.

**Animals, treatments, pasture management and supplementation**

Forty-eight ½ Angus ½ Nelore heifers with an average initial weight of approximately

276.70 ± 20.1 kg and an average age of nine months were used. The experimental design was a randomized block with three treatments and four replicates per treatment (Table 2).

**Table 2**  
**Paddocks and treatments in the experimental area. Andradina - São Paulo, Brazil**

Paddocks	Area (ha)	Production system
A	1.88	Conventional System (CS)
B	1.95	Conventional System (CS)
C	1.81	Conventional System (CS)
D	2.04	Conventional System (CS)
E	2.75	Integrated Livestock-Forest, with trees in triple rows (ILF-3L)
F	2.03	Integrated Livestock-Forest, with trees in simple lines (ILF-1L)
G	2.12	Integrated Livestock-Forest, with trees in simple lines (ILF-1L)
H	1.97	Integrated Livestock-Forest, with trees in triple rows (ILF-3L)
I	2.33	Integrated Livestock-Forest, with trees in simple lines (ILF-1L)
J	2.13	Integrated Livestock-Forest, with trees in triple rows (ILF-3L)
K	2.39	Integrated Livestock-Forest, with trees in simple lines (ILF-1L)
L	2.31	Integrated Livestock-Forest, with trees in triple rows (ILF-3L)
Total	25.71	

ILF-1L: eucalyptus trees planted in simple lines, the distance between each strip of eucalyptus from 17 to 21 m and the distance between plants of 2 m, with density of 196 trees ha<sup>-1</sup>; ILF-3L: eucalyptus trees planted in triple rows, the distance between the eucalyptus bands from 17 to 21 m, the distance between 2 m plants and the distance between 3 m eucalyptus lines with a density of 448 trees ha<sup>-1</sup>.

The adopted grazing method was continuous stocking with a variable stocking rate, using the *put-and-take* method (Mott & Lucas, 1952). In each paddock, six *tester* animals and a variable number of regulators were used, according to the need to adjust the stocking rate to maintain the management goal, with an average lawn height of 30 cm, which is within the range (20-40 cm) considered as optimal pasture conditions (S. C. Silva, 2004). The height

conditions of the forage were monitored using a ruler graduated in centimeters (cm), and the distance between the curvature of the highest leaf at the sampling point and the soil was measured (Hodgson, 1990) at average intervals of 14 days, with an average sampling number of 100 points per paddock.

The animals received supplementation according to the time of year, following the availability, quality of forage, and

animals' needs. During the period from June to November 2017, a protein mineral supplement was provided, with an intake of 0.1% live weight (LW) with 40% crude protein (CP) and 32% total digestible nutrients (TDN). In the period from December 2017 to March 2018, a supplement for the wet period was provided, with a consumption of 0.1% LW (20% CP and 50% TDN). In the period from April to June 2018, the animals were finished with Potensal® feed supplied daily and an intake of 0.7% of LW (16% CP and 75% TDN).

### *Slaughter and collection of samples*

At the end of the established continuous grazing period, the animals underwent solid fasting for approximately 16 hours with free access to water. Later, they were weighed ( $432.8 \pm 31.30$  kg) and transported to the slaughterhouse. The animals were slaughtered humanely, in a commercial slaughterhouse, following the establishment's operational flow, as well as its good manufacturing practices. The animal was restrained and stunned by a captive bolt pistol and subsequently bled by jugular vein section. After slaughter, the carcass halves were identified, washed, and stored in a cold chamber (Temperature =  $0.0 \pm 2.0^\circ\text{C}$ ) for 24 hours.

Subsequently, a section between the 8th and 13th thoracic vertebrae of the *Longissimus thoracis* muscle was performed and then transported to the Meat Quality Laboratory of the College of Agricultural and Technological Sciences, Dracena, where cross-cuts approximately 2.5 cm wide were made with a band saw (Model 255, Beccaro, Toronto, ON, Canada). These cross-cuts were later used in the analysis. Then, each cut was

vacuum packed ( $18 \mu$ ) in a JETVAC® wrapper (200-B, Selovac, São Paulo, Brazil) and frozen ( $-20^\circ\text{C}$ ) until the time of analysis. The analyses of Enterobacteriaceae, *Salmonella* ssp., fungi, yeasts, and coliforms were performed immediately after slaughter. On the other hand, pH, lipid oxidation, meat color, total bacterial count, and psychrotrophic bacteria analyses were carried out according to a  $3 \times 5$  factorial scheme, with three treatments (CS, ILF-1L, and ILF-3L) and five periods of frozen storage (30, 60, 90, 180, and 360 days).

### *Hydrogenionic potential (pH) of meat and meat color*

The pH was determined according to the method described by Beltrán et al. (1997) using a pH meter (Model HI 99163, Brand HANNA, Woonsocket - USA) with combined electrodes for reading in triplicate with perforations at three points of each repetition 24 hours postmortem. The equipment was calibrated with pH 4.1 and 7.1 buffer solutions before use.

The meat color was determined by taking readings at three random points on the surface of the *longissimus thoracis* muscle cut of each sample, using a portable spectrophotometer (CR-410-Konica Minolta, Camera Co., Ltd. Osaka, Japan) with D65 illuminant, an 8.0-mm opening diameter, and a  $10^\circ$  observation angle (American Meat Science Association [AMSA], 2012), previously calibrated with a white standard according to the manufacturer's instructions. The CIELAB system was considered through readings of light reflectance in three dimensions:  $L^*$  (Lightness),  $a^*$  (Redness), and  $b^*$  (Yellowness), according to the methodology described by Honikel (1998).



The values of Chroma ( $C^*$ ), Hue angle ( $H^*$ ), and global color changes ( $\Delta E$ ) were determined according to MacDougal (1994) and the oxymyoglobin and metmyoglobin content (O/M) present on the meat surface were determined according to Olivo and Shimokomaki (2001), using the coordinates of redness ( $a^*$ ) and yellowness ( $b^*$ ), obtained in colorimetric determinations with the following formulas:  $C^* = ((a^*)^2 + (b^*)^2)^{0.5}$ ;  $H^* = \tan^{-1}(b^*/a^*)$ ;  $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5}$ ;  $O/M = (a^*/b^*)$ .

### Meat oxidation analysis

In the assessment of lipid oxidation, the equivalent oxidation of malonaldehyde was observed using the TBARS (Thiobarbituric Acid Reactive Substance) methodology according to Wyncke (1970). Ten-gram meat samples were weighed, thawed in the refrigerator for eight hours, and homogenized for two minutes using an Ultra-Turrax mixer. Subsequently, 50 mL of 7.5% trichloroacetic acid solution was added. This mixture was then filtered, and a 5.0 mL aliquot was mixed with 5.0 mL of TBA solution (0.020 mol/L) and placed in a water bath (100°C) for 30 minutes. The absorbance of the samples was measured at 532 nm with a spectrophotometer (FEMTO 600, São Paulo, Brazil) in duplicate and the result expressed in milligrams of malonaldehyde (MDA) per kilogram of meat using a standard curve made with 1,1,3,3-tetraethoxypropane.

### Microbiological analysis

Microbiological analysis was based on the "Compendium of Methods for the Microbiological Examination of Foods"

(Downes & Ito, 2001). The following values were evaluated: total bacterial counts, psychrotrophic bacteria, Enterobacteriaceae, *Salmonella* spp., filamentous fungi, yeasts, total coliforms, and thermotolerant coliforms. The samples were prepared by removing aliquots of approximately 10 g from the meat sample, which were then homogenized in 90 mL 0.1% sterile peptone saline. Then, serial 10-fold dilutions were made in 0.1% sterile peptone saline until a  $10^{-5}$  dilution was reached.

From each sample dilution, 1.0 mL was deposited in a sterile Petri dish, and then approximately 15 mL of Plate Count Agar was added for total bacterial counts and psychrotrophic bacteria, while Crystal Violet Bile Dextrose Agar was used for the analysis of Enterobacteriaceae, fused and cooled to a temperature of around 45°C. The inoculum was mixed with the culture medium using circular movements. After complete solidification of the medium, the plates were inverted and incubated at 32°C for 48 hours for Total Bacteria Counts and Enterobacteriaceae and at 7.0°C for 10 days for psychrotrophic bacteria counts. Plaques containing between 25 and 250 colonies were selected for colony counts.

In the *Salmonella* ssp. research, 225 mL of buffered water and 25 g of sample were combined and stored at a temperature of 35°C for 24 hours. Then, 1.0 mL aliquots of this suspension were transferred to either 100 mL of Selenite Cystine Broth or 10 mL of Tetrathionate Broth, and both cultures were incubated at 35°C for 24 hours. After this period, the bacteria were plated on Petri dishes containing Agar Salmonella-Shigella (differential plating method), and the inverted plates were incubated at 35°C for

24 hours. Filamentous fungi and yeasts were determined by plating in acidified Potato Glucose Agar, in plates incubated at 21°C for five days.

The total and thermotolerant coliform counts were determined according to the methodology suggested by N. Silva et al. (2001), which was based on Normative Instruction No. 62, of August 26, 2003, issued by (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2003). Twenty-five grams of meat were placed in 225 mL of 0.1% sterile peptone water. After homogenization, a  $10^{-1}$  dilution was obtained, and then serial 10-fold dilutions were performed until a  $10^{-3}$  dilution was reached. For the presumptive test, 1.0 mL aliquots of each dilution were inoculated into tubes with lactate broth and incubated in an oven at 35°C for 24-48 hours. In the confirmatory test, lactate tubes that showed gas production were transferred, one per culture, to tubes with Bright Green broth for total coliform counts, and also, one per culture, to tubes with *Escherichia coli* broth. Bright Green tubes were incubated at 35°C for 24-48 hours and *Escherichia coli* broth tubes at 45°C for 24-48 hours. The results of the microbiological tests were expressed as the Most Probable Number per gram (MPN/g) of sample.

### *Form of data analysis*

The data were analyzed by SAS 9.4 (Statistical Analysis System Institute [SAS Institute], 2010). The normality of the data was confirmed by the univariate normal procedure and the Shapiro-Wilk test ( $W \geq 0.90$ ). Subsequently, the data were analyzed using the PROC MIXED procedure. Statistically significant effects were considered when  $P$  was  $< 0.05$  by the Tukey test (system), and regression analysis was performed between the freezing days (30, 60, 90, 180, and 360 days).

## **Results and Discussion**

No interactions between system  $\times$  freezing days ( $P > 0.05$ ) were observed in any of the variables analyzed. Among the treatments, there were also no differences in pH, lipid oxidation (MDA), color, total bacterial count (TBC), and psychrotrophic bacteria ( $P > 0.05$ ; Table 3).

**Table 3**  
Hydrogenionic potential, color, lipid oxidation (malonaldehyde content - MDA) and microbiological quality of the meat of Angus-Nelore heifers in conventional systems (CS) and Integrated Livestock-Forest (ILF) with a density of 196 trees ha<sup>-1</sup> (ILF-1L) and 448 trees ha<sup>-1</sup> (ILF-3L) and subjected to different periods of freezing

Systems	Days	pH	L*	a*	b*	C*	H*	O/M	MDA	TBC	PSI
CS		5.91	36.20	17.24	7.82	18.95	24.25	2.26	0.579	2.70	1.95
ILF-1L		5.89	36.18	17.42	7.86	19.13	23.91	2.25	0.590	2.73	1.99
ILF-3L		5.90	35.94	17.49	7.74	19.16	23.95	2.33	0.588	2.69	2.09
	30	5.74	35.02	15.54	6.74	16.95	23.13	2.36	0.246	2.76	2.08
	60	5.80	36.27	17.35	7.34	18.87	23.32	2.45	0.414	2.77	2.20
	90	5.84	35.95	17.93	8.16	19.72	24.27	2.23	0.501	2.60	1.93
	180	5.97	35.75	18.02	8.09	19.77	24.30	2.26	0.504	2.66	1.88
	360	6.14	37.56	18.08	8.71	20.09	25.17	2.11	1.264	2.72	1.97
						<i>P-value</i>					
Systems		0.491	0.821	0.743	0.891	0.854	0.684	0.166	0.821	0.907	0.119
Days		<0.001 <sup>1</sup>	0.499	<0.001 <sup>2</sup>	<0.001 <sup>3</sup>	<0.001 <sup>4</sup>	0.002 <sup>5</sup>	<0.001 <sup>6</sup>	<0.001 <sup>7</sup>	0.567	0.062
Systems*Days		0.476	0.242	0.372	0.460	0.379	0.516	0.452	0.615	0.852	0.753
SE		0.012	0.205	0.154	0.110	0.180	0.181	0.021	0.024	0.036	0.030

pH = Hydrogen potential; L\* = Lightness; a\* = Redness; b\* = yellowness; C\* = Chroma; H\* = Hue angulo; MDA = Malonaldehyde (mg/kg); CTB = Total bacteria count (log CFU/g of meat); PSI = Psychrotrophic bacteria (log CFU/g of meat); SE = Standard error. Systems: Averages followed by lowercase and distinct letters, in the column, they differ by the Tukey test (P<0.05). Days: Regression Analysis.

1Y = 5.72374260 + 0.00120289x (R<sup>2</sup> = 0.99);

2Y = 16.6583629 + 0.00503915x (R<sup>2</sup> = 0.96);

3Y = 6.56453885 + 0.01438015x - 0.00002371x<sup>2</sup> (R<sup>2</sup> = 0.96);

4Y = 16.7613241 + 0.03074655x - 0.00006045x<sup>2</sup> (R<sup>2</sup> = 0.97);

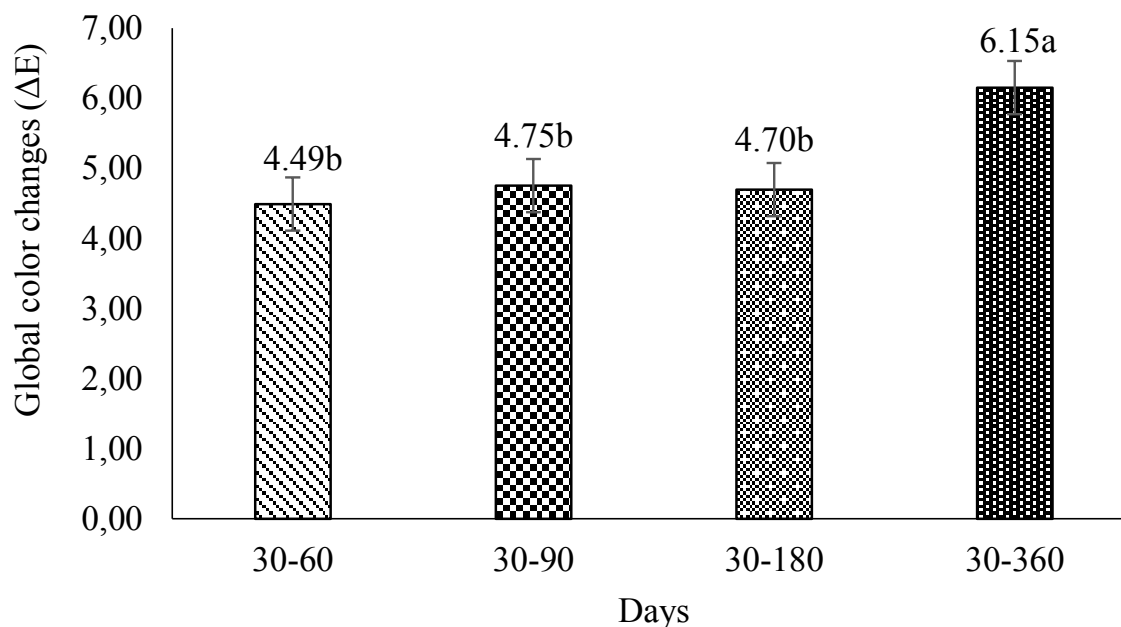
5Y = 23.2153268 + 0.00571214x (R<sup>2</sup> = 0.94);

6Y = 2.40001798 - 0.00082854x (R<sup>2</sup> = 0.73);

7Y = 0.17725783 + 0.00286646x (R<sup>2</sup> = 0.92).

On the other hand, there was an effect of the number of storage days on pH,  $a^*$ ,  $b^*$ ,  $C^*$ ,  $H^*$ , and lipid oxidation, which increased in value as the storage time increased, even though the meat was frozen ( $P < 0.05$ ; Table 3).

Moreover, at 360 days of freezing, the global changes in the color of the meat showed the greatest change compared to other periods of freezing ( $P < 0.05$ ; Figure 1).



**Figure 1.** Global color changes ( $\Delta E_{30-60}$ ,  $\Delta E_{30-90}$ ,  $\Delta E_{30-180}$  and  $\Delta E_{30-360}$ ) in meat samples from Angus-Nelore heifers ( $P = 0.08$ ;  $SE = 0.189$ ; Scale: 0-0.2 corresponds to changes imperceptible to the human eye; 0.2-0.5 very little perceptible; 0.5-1.5 little noticeable; 1.5-3.0 evident perceptions; 3.0-6.0 very evident perceptions; 6-12 very clear perception; 12-14 easily perceived; Prändl et al., 1994).

The values of lipid oxidation increased over the storage period ( $P < 0.05$ ; Table 3). On the other hand, there was no effect of freezing storage on the microbiological quality assessed by counting bacteria and psychrotrophic bacteria ( $P > 0.5$ ; Table 3).

In the analysis of Enterobacteriaceae, Salmonella spp., fungi, and yeasts in heifer meat according to the evaluated systems (CS,

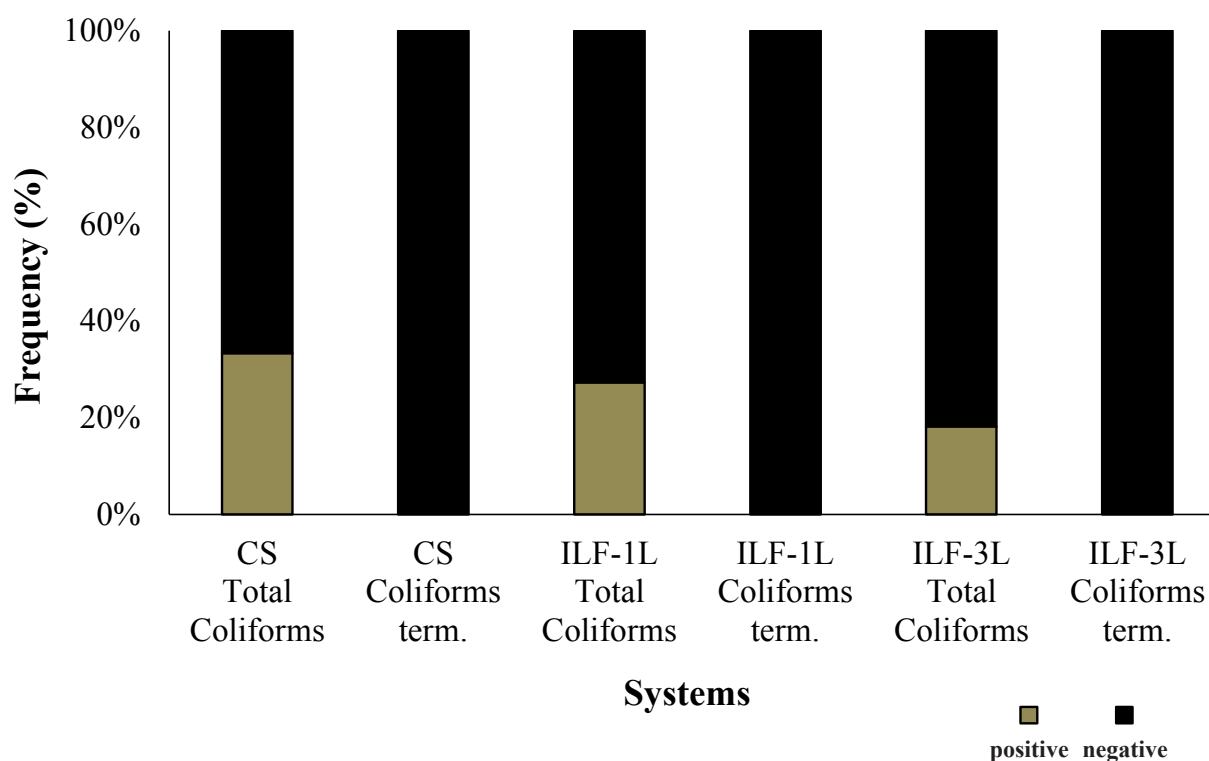
ILF-1L, and ILF-3L), there was no difference between the different treatments ( $P > 0.05$ ; Table 4). Regarding total coliforms, there was a higher positive frequency in the meat of animals kept in the conventional system (33.3%), followed by the ILF-1L (27.3%) and ILF-3L (18.2%) systems, and an absence of thermotolerant coliforms (Figure 2).

Table 4

Analysis of Enterobacteriaceae, *Salmonella ssp.*, fungi and yeasts of the meat of Angus-Nelore heifers in conventional systems (CS) and Integrated Livestock-Forest (ILF) with a density of 196 trees ha<sup>-1</sup> (ILF-1L) and 448 trees ha<sup>-1</sup> (ILF-3L)

Variables	CS	ILF-1L	ILF-3L	P-value	SE
Enterobacteriaceae	1.63	1.64	1.72	0.579	0.033
<i>Salmonella ssp.</i>	Absent	Absent	Absent	-	-
Fungi	0.96	1.10	1.07	0.735	0.087
Yeasts	0.47	0.49	0.67	0.417	0.078

Enterobacteriaceae, fungi and yeasts unit = (log CFU/g of meat); *Salmonella ssp.* unit = grams; SE = Standard error. Averages followed by lowercase and distinct letters, in the column, they differ by the Tukey test (P<0.05).



**Figure 2.** Frequency of positive and negative results for total and thermotolerant coliforms (positive: coliforms > 3 MPN/g - negative: coliforms < 3 MPN/g) of the meat of Angus-Nelore heifers in conventional systems (CS) and Integrated Livestock-Forest (ILF) with a density of 196 trees ha<sup>-1</sup> (ILF-1L) and 448 trees. ha<sup>-1</sup> (ILF-3L).

Although trees promoted a microclimate favorable to animal welfare during the experimental period (Table 1), they did not influence meat quality, since meat quality in systems with different tree densities (ILF-1L and ILF-3L) did not differ from that in the conventional system (CS). The pH values confirm this fact, indicating normal patterns of development of the physical and chemical characteristics responsible for meat quality since they are below the limit ( $\text{pH} > 6.0$ ) perceived by the animal as heat stress (Pedrão et al., 2015). In addition, export-wise, the values found were still below the pH limit defined for the export of meat to the European Union ( $\text{pH} = 5.9$ ; according to Circular 192/98/DCI/DIPOA).

The absence of differences in pH between treatments also justifies the comparative results obtained for meat color variables ( $a^*$ ,  $b^*$ , and  $L^*$ ) since color changes do not occur when there are no biochemical changes (Gomide & Ramos, 2017). Likewise, the chroma ( $C^*$ ), the Hue angle ( $H^*$ ), and the oxymyoglobin and metmyoglobin contents (O/M) did not differ between treatments due to their determination using the  $L^*$ ,  $a^*$ , and  $b^*$  coordinates. In addition, color-wise, the values for lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) were within the standards proposed for beef, which vary from 33.2 to 40.0, 11.1 to 24.02, and 6.1 to 17.59, respectively (Gomide & Ramos, 2017).

Regarding lipid oxidation, the absence of differences between treatments was expected since the increase in free radical production is related to heat stress conditions in animals. In this case, free radicals are not balanced by the antioxidant protection system; this condition is called oxidative stress, which manifests itself

through its effect on lipid oxidation (Hassan et al., 2020). In the present study, there was no evidence of stress in animals, as appropriate pH values were obtained for meat in all evaluated treatments, a fact that also did not compromise lipid oxidation.

As for the evaluation of microbiological quality in different treatments, it was observed that both the total and psychrotrophic bacterial counts were below the values that classify meat as contaminated. According to Normative Instruction No. 60 of the Ministério da Saúde - Agência Nacional de Vigilância Sanitária (ANVISA, 2019), all microorganisms were within the maximum limit of  $10^6$  CFU/g (6 log CFU/g) considered acceptable. This value must be related to the pH since a higher pH is known to be associated with increased microbial counts in meat, and the average values observed were adequate for its maintenance (Alcantara et al., 2012). Although there were no changes in any of the quality variables analyzed between the systems, it is important to note that this does not suggest that the use of trees in the pasture does not contribute to the welfare of the animals. On the contrary, the tree component should be encouraged, as it offers quality meat as much as a conventional system does.

Regarding the storage period, previous studies explain the pH variance during the storage period by enzymatic action, that is, the storage process increases the osmotic pressure of the medium as a result of the degradation of proteins into smaller molecules and the intramolecular reorganization of these proteins, which determines changes in their electrical charges, thus increasing the pH (Fernandes et al., 2012).

As for the color, it was observed that the linear increase in pH caused the meat to darken as the freezing storage period increased. This fact is evidenced by the increase in  $b^*$ , which indicates an increase in the content of metmyoglobin in meat, as well as by the reduction in the values of O/M content ( $P < 0.05$ ; Table 3), i.e., higher results for this variable indicate a higher concentration of oxymyoglobin, while lower values suggest higher concentrations of metmyoglobin (Tomasevic et al., 2019).

The increasing metmyoglobin content over the storage period can still be explained by the quadratic and linear effects of  $C^*$  and  $H^*$ , respectively, which do not present value patterns. However, it is suggested that the increase in the values of these variables is usually accompanied by the process of discoloration of the meat, that is, discoloration occurs with lower concentrations of oxymyoglobin, which is responsible for giving the meat a bright red color (Tomasevic et al., 2019). Furthermore, at 360 days of freezing, the global changes in the color of the meat were the greatest compared to other periods of freezing. For this period, the changes were classified as clearly perceptible by the human eye (Prändl et al., 1994).

Regarding the increase in lipid oxidation over the storage period, the results suggest that the reduction in temperature alone was not sufficient to prevent lipid oxidation, which occurs even during storage of frozen foods, as freezing does not stop oxidative reactions (Leão et al., 2017). In addition, lipids are oxidized over time, especially those that make up polyunsaturated fatty acids, which are more sensitive to oxidation (Geay et al., 2001).

On the other hand, analyses of total counts of bacteria and psychrotrophic bacteria did not show any effect as the period of frozen storage increased, even with the increase in the pH of the meat, which when elevated, making beef an ideal culture medium for many microorganisms (Forsythe, 2013). The results obtained indicate the efficiency of freezing in stopping microbial growth over different storage time periods, proving that freezing can be a good way of extending the shelf life of meat.

The absence of difference in the analyses of Enterobacteriaceae, *Salmonella* spp., fungi, and yeasts of heifer meat depending on the system (CS, ILF-1L, and ILF-3L) is possibly due to the discovered results for pH since the pH of the meat of the animals has a direct influence on the microbiological quality of the meat. As the pH remained the same between treatments, the absence of differences between microbiological variables of the meat was also expected. Regarding total coliforms, the majority of frozen samples showed no contamination. Those that showed total coliform contamination gave negative results in the thermotolerant coliform test. Thus, the microbiological evaluation showed that all samples remained adequate for consumption throughout the storage period.

## Conclusion

The physical and microbiological quality and oxidative stability of the meat are not affected by the tree component in the pasture. Meat undergoes changes in pH, color, and lipid oxidation as the frozen storage time increases, regardless of the production system. However, these changes

do not affect the microbiological quality for up to 360 days of storage, making the meat appropriate for consumption.

## Acknowledgment

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and Fapesp (Process 2019/20039-5). The authors declare no conflicts of interest.

## References

- Agência Nacional de Vigilância Sanitária (2019). Instrução Normativa N°60, de 23 de Dezembro de 2019. [chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://cvs.saude.sp.gov.br/zip/U\\_IN-MS-ANVISA-60\\_231219.pdf](https://cvs.saude.sp.gov.br/zip/U_IN-MS-ANVISA-60_231219.pdf)
- Alcantara, M. de, Morais, I. C. L. de, Matos, C. de, & Souza, O. da C. C. de. (2012). Principais microrganismos envolvidos na deterioração das características sensoriais de derivados cárneos. *Revista Brasileira de Higiene e Sanidade Animal*, 6(1), 1-20. doi: 10.5935/rbhsa.v6i1.15
- Alvares, C. A., Stape, J. L., Sentelhas, P. C., Moraes Gonçalves, J. L. de, & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, 22(6), 711-728. doi: 10.1127/0941-2948/2013/0507
- American Meat Science Association (2012). *Meat color measurement guidelines*. AMSA. [https://meatscience.org/docs/default-source/publications-resources/hot-topics/2012\\_12\\_meat\\_clr\\_guide.pdf?sfvrsn=d818b8b3\\_0](https://meatscience.org/docs/default-source/publications-resources/hot-topics/2012_12_meat_clr_guide.pdf?sfvrsn=d818b8b3_0)
- Barbosa, F. A., Costa, P. M., Andrade, V. J. de, Maia Filho, G. H. B., Maciel, I. C. de F., & Lopes, S. Q. (2015). Avaliação econômica e produtiva dos sistemas integrados de lavoura-pecuária-floresta. *Caderno De Ciências Agrárias*, 7(1), 151-165. <https://periodicos.ufmg.br/index.php/ccaufmg/article/view/2847>
- Beltrán, J. A., Jaime, I., Santolaria, P., Sañudo, C., Albertí, P., & Roncalés, P. (1997). Effect of stress-induced high post-mortem pH on protease activity and tenderness of beef. *Meat Science*, 45(2), 201-207. doi: 10.1016/s0309-1740(96)00103-9
- Domiciano, L. F., Mombach, M. A., Carvalho, P., Silva, N. M. F., Pereira, D. H., Cabral, L. S., Lopes, L. B., & Pedreira, B. C. (2016). Performance and behaviour of Nelore steers on integrated systems. *Animal Production Science*, 58(5), 920-929. doi: 10.1071/an16351
- Domínguez, A., Torner, N., Ruiz, L., Martínez, A., Bartolomé, R., Sulleiro, E., Teixidó, A., & Plasencia, A. (2007). Foodborne Salmonella-caused outbreaks in Catalonia (Spain), 1990 to 2003. *Journal of Food Protection*, 70(1), 209-213. doi: 10.4315/0362-028x-70.1.209
- Downes, F. P., & Ito, K. (2001). *Compendium of methods for the examination of foods* (2nd ed.). APHA.
- Empresa Brasileira de Pesquisa Agropecuária (2013). *Sistema brasileiro de classificação de Solos* (3a ed.). EMBRAPA Solos. <http://livimagens.sct.embrapa.br/amostras/00053080.pdf>
- Fernandes, R. de P. P., Freire, M. T. de A., Guerra, C. C., Carrer, C. da C., Balieiro, J. C. de C., & Trindade, M. A. (2012). Estabilidade



- físico-química, microbiológica e sensorial de carne ovina embalada a vácuo estocada sob refrigeração. *Ciência Rural*, 42(4), 724-729. doi: 10.1590/S0103-84782012000400025
- Food and Agriculture Organization of the United Nations (2016). *Integrated Crop-Livestock Systems* (ICLS). FAO. <http://www.fao.org/agriculture/crops/core-themes/theme/spi/scpihome/managing-ecosystems/integrated-crop-livestock-systems/en/>
- Forrest, J. C., Aberle, E. D., Hedrick, H. B., Judge, M. D., & Merkel, R. A. (1979). *Fundamentos de ciencia de la carne*. Acibia.
- Forsythe, S. J. (2013). *Microbiologia da segurança dos alimentos*. Artmed.
- Geay, Y., Bauchart, D., Hocquette, J.-F., & Culioli, J. (2001). Effect of nutritional factors on biochemical, structural and metabolic characteristics of muscles in ruminants, consequences on dietetic value and sensorial qualities of meat. *Reproduction Nutrition Development*, 41(1), 1-26. doi: 10.1051/rnd:2001108
- Gomide, L. A. M., & Ramos, E. M. (2017). *Avaliação da qualidade de carnes: fundamentos e metodologias*. Editora UFV.
- Gomide, L. A. M., Ramos, E. M., & Fontes, P. R. (2014). *Tecnologia de abate e tipificação de carcaças*. Editora UFV.
- Gregory, N. G. (2010). How climatic changes could affect meat quality. *Food Research International*, 43(7), 1866-1873. doi: 10.1016/j.foodres.2009.05.018
- Hassan, A. H. A., Hozzein, W. N., Mousa, A. S. M., Rabie, W., Alkhalifah, D. H. M., Selim, S., & AbdElgawad, H. (2020). Heat stress as an innovative approach to enhance the antioxidant production in *Pseudooceanicola* and *Bacillus* isolates. *Scientific Reports*, 10(1), 15076 doi: 10.1038/s41598-020-72054-y
- Hodgson, J. (1990). *Grazing management: science practice*. Longman Scientific & Technical.
- Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science*, 49(4), 447-457. Doi: 10.1016/s0309-1740(98)00034-5
- Janni, M., Gullì, M., Maestri, E., Marmiroli, M., Valliyodan, B., Nguyen, H. T., & Marmiroli, N. (2020). Molecular and genetic bases of heat stress responses in crop plants and breeding for increased resilience and productivity. *Journal of Experimental Botany*, 71(13), 3780-3802. doi: 10.1093/jxb/eraa034
- Leão, L. L., Oliveira, F. S., Souza, R. S., Farias, P. K. S., Fonseca, F. S. A. da, Martins, E. R., & Souza, R. M. de. (2017). Uso de antioxidantes naturais em carnes e seus subprodutos. *Caderno de Ciências Agrárias*, 9(1), 94-100. doi: <https://periodicos.ufmg.br/index.php/ccaufmg/article/view/2951>
- Lu, Z., He, X., Ma, B., Zhang, L., Li, J., Jiang, Y., Zhou, G., & Gao, F. (2017). Chronic heat stress impairs the quality of breast-muscle meat in broilers by affecting redox status and energy-substance metabolism. *Journal of Agricultural and Food Chemistry*, 65(51), 11251-11258. doi: 10.1021/acs.jafc.7b04428

- MacDougall, D. B. (1994). Colour meat. In A. M. Pearson, & T. R. Dutson (Orgs.), *Quality attributes and their measurement in meat, poultry and fish products* (Cap. 9, pp. 79-93). Boston, MA: Advances in Meat Research Series. Blackie Academic & Professional.
- Melo, A. F., Moreira, J. M., Ataídes, D. S., Guimarães, R. A. M., Loiola, J. L., & Oliveira, R. Q. de. (2016). Fatores que influenciam na qualidade da carne bovina: revisão. *Pubvet*, 10(10), 785-794. doi: 10.22256/pubvet.v10n10.785-794
- Ministério da Agricultura, Pecuária e Abastecimento (2003). *Métodos oficiais para análise de produtos de origem animal*. [https://www.gov.br/agricultura/pt-br/assuntos/lfda/legislacao-metodos-da-rede-lfda/poa/metodos\\_oficiais\\_para\\_analise\\_de\\_produtos\\_de\\_origem\\_animal-\\_1a\\_ed-\\_2022\\_assinado.pdf](https://www.gov.br/agricultura/pt-br/assuntos/lfda/legislacao-metodos-da-rede-lfda/poa/metodos_oficiais_para_analise_de_produtos_de_origem_animal-_1a_ed-_2022_assinado.pdf)
- Mott, G. O., & Lucas, H. L. (1952). The design conduct and interpretation of grazing trials on cultivated and improved pastures. *Proceedings of the International Grassland Congress*, State College, PA, USA, 6.
- Oliveira, A. F., Menezes, G. L., Gonçalves, L. C., Araújo, V. E., Ramirez, M. A., Guimarães, R. G., Jr., Jayme, D. G., & Lana, A. M. Q. (2022). Pasture traits and cattle performance in silvopastoral systems with Eucalyptus and Urochloa: systematic review and meta-analysis. *Livestock Science*, 262(2), 104973. doi: 10.1016/j.livsci.2022.104973
- Olivo, R., & Shimokomaki, M. (2001). *Carnes: no caminho da pesquisa*. Imprint.
- Pedraço, M. R., Kato, T., Soares, A. L., Ida, E. I., Coró, F. A. G., Grespan, M., Paião, F., & Shimokomaki, M. (2015). Influence of cooling on the glycolysis rate and development of PSE (Pale, Soft, Exudative) meat. *Brazilian Archives of Biology and Technology*, 58(2), 272-277. doi: 10.1590/s1516-8913201400154
- Porfírio-da-Silva, V., Medrado, M. J. S., Nicodemo, M. L. F., & Dereti, R. M. (2010). *Arborização de pastagens com espécies florestais madeireiras: implantação e manejo*. EMBRAPA Florestas.
- Portugal, T. B., Carvalho, P. C. F., Campos, B. M., Szymczak, L. S., Savian, J. V., Zubieta, A. S., Souza, W. S., Fº., Rosseto, J., Bremm, C., Oliveira, L. B., Moraes, A., Bayer, C., & Monteiro, A. L. G. (2023). Methane emissions and growth performance of beef cattle grazing multi-species swards in different pesticide-free integrated crop-livestock systems in southern Brazil. *Journal of Cleaner Production*, 414(4), 137536. doi: 10.1016/j.jclepro.2023.137536
- Prändl, O., Fischer, A., Schmidhofer, T., & Sinell, H. J. (1994). *Tecnologia e higiene de la carne*. Acribia.
- Silva, N., Junqueiro, V. C. A., & Silveira, N. F. A. (2001). *Manual de métodos de análise microbiológica de alimentos* (2a ed.). Varela.
- Silva, S. C. (2004). Fundamentos para o manejo do pastejo de plantas forrageiras dos gêneros *Brachiaria* e *Panicum*. *Anais do Simpósio sobre Manejo Estratégico da Pastagem*, Viçosa, MG, Brasil.
- Statistical Analysis System Institute (2010). *SAS User's guide: Statistics*. Version 9.4. SAS Institute.

- Tomasevic, I., Tomovic, V., Milovanovic, B., Lorenzo, J., Đorđević, V., Karabasil, N., & Djekic, I. (2019). Comparison of a computer vision system vs. traditional colorimeter for color evaluation of meat products with various physical properties. *Meat Science*, 148(2), 5-12. doi: 10.1016/j.meatsci.2018.09.015
- Universidade Estadual de Campinas (2012). *Clima dos municípios paulistas*. Centro de Pesquisas Meteorológicas e Climáticas Aplicadas a Agricultura. UNICAMP. [http://www.cpa.unicamp.br/outras-informacoes/clima\\_muni\\_024.html](http://www.cpa.unicamp.br/outras-informacoes/clima_muni_024.html).
- Van der Pol, A., Van Gilst, W. H., Voors, A. A., & Van der Meer, P. (2019). Treating oxidative stress in heart failure: past, present and future. *European Journal of Heart Failure*, 21(4), 425-435. doi: 10.1002/ehhf.1320
- Van Raij, B., Cantarella, H., Quaggio, J. Á., & Furlani A. M. C. (1997). *Recomendações de adubação e calagem para o Estado de São Paulo* (2a ed. rev. e atual.). Instituto Agrônômico/Fundação IAC.
- Wyncke, W. (1970). Direct determination of the thiobarbituric acid value in trichloroacetic acid extracts of fish as a measure of oxidative rancidity. *Fette, Seifen, Anstrichmittel*, 72, 1084-1087. doi: 10.1002/lipi.19700721218

