

# Identification of the production system of beef cattle by the stable isotope analysis

**Abstract** – The objective of this work was to evaluate the potential of the stable isotope technique to characterize beef cattle production systems in tropical conditions. For this, carbon and nitrogen stable isotopes were identified in non-defatted and defatted bovine muscles. A total of 45 cattle were evaluated in three production systems: pasture, conventional feedlot, and young beef bull feedlot (n = 15 per system). Samples from the *Trapezius cervicis*, *Longissimus dorsi*, and *Semitendinosus* muscles were collected to determine the isotopic composition of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . The isotopic data of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of non-defatted and defatted muscles were subjected to the principal component analysis (PCA) and to the discriminant analysis (DA). The PCA allowed separating the three production systems based on the results obtained for the non-defatted and defatted muscles. A correct global classification rate of 100% and a cross-validation rate of 100% were obtained with the DA. The carbon and nitrogen isotopic ratio of non-defatted and defatted muscles allows for the precise identification of beef cattle production systems in tropical conditions.

**Index terms:** muscle tissue, Nelore, production system, traceability.

## Identificação do sistema de produção de bovinos de corte pela análise de isótopos estáveis

**Resumo** – O objetivo deste trabalho foi avaliar o potencial da técnica de isótopos estáveis para caracterizar sistemas de produção de carne bovina em condições tropicais. Para tanto, foram identificados isótopos estáveis de carbono e nitrogênio em músculos de bovinos não desengordurados e desengordurados. Avaliaram-se 45 bovinos criados em três sistemas de produção: a pasto, confinamento convencional e confinamento de novilhos superprecoces (n= 15 por sistema). Amostras dos músculos *Trapezius cervicis*, *Longissimus dorsi* e *Semitendinosus* foram coletadas para determinar a composição isotópica de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$ . Os dados isotópicos de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  em músculos não desengordurados e desengordurados foram submetidos à análise de componentes principais (ACP) e à análise discriminante (AD). A ACP permitiu a separação dos três sistemas de produção com base nos resultados obtidos para os músculos não desengordurados e desengordurados. Obtiveram-se uma taxa de classificação correta geral de 100% e uma taxa de validação cruzada de 100% com a AD. A razão isotópica de carbono e nitrogênio em músculos não desengordurados e desengordurados permite identificar com precisão os sistemas de produção de bovinos de corte em condições tropicais.

**Termos para indexação:** tecido muscular, Nelore, sistema de produção, rastreabilidade.

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## Introduction

The Brazilian cattle industry produces around 10 million tons of beef, of which approximately 20.8% are exported to other countries, following rigorous quality standards (ABIEC, 2020). However, the production systems in Brazil are considered highly heterogeneous, which triggers several problems, especially related to health issues (Vinholis et al., 2016). In the country, Sistema Brasileiro de Identificação Individual de Bovinos e Búfalos (Sisbov) is the official system used for the traceability of bovine and bubaline from birth to slaughter, supporting decision making regarding the quality of national and imported cattle. However, this traceability is currently based on ear tags, life numbers, and electronic devices or tattoos, which can be lost or mislabeled (Guo et al., 2010). Therefore, a reliable and scientific system is urgently needed to characterize beef production in Brazil.

The possibility of determining how an animal was produced and where it was bred is an advance for the traceability and certification system in the country, meeting one of the requirements of the consumer markets to ensure the characteristics of the obtained product based on how it was handled before slaughter and also on its origin. A technique with potential to assess food authenticity is the stable isotope analysis, which allows to investigate food sources that are isotopically distinct (DeNiro & Epstein, 1978).

The stable isotope and/or multielement analysis is an increasingly accepted tool for tracing the geographical origin of production animals, as well as for characterizing and differentiating their diet regime (González-Martin et al., 1999; Piasentier et al., 2003; Guo et al., 2010; Zhao et al., 2013). In Europe, stable isotopes are used to determine, according to production area and method, the quality of foods, in order to correctly label them, mainly those certified as having “protected designation of origin”, “protected geographical indication”, and “guaranteed traditional specialty” (Camin et al., 2017). In China, the technique is used to support the inspection and quality control of products in the markets (Chen et al., 2017).

The objective of this work was to evaluate the potential of the stable isotope technique to characterize beef cattle production systems in tropical conditions.

## Materials and Methods

The guidelines followed for animal use were those established by the ethical committee for animal research (CEUA) of Universidade do Estado de São Paulo (UNESP), protocol number 169/2010-CEUA. A total of 45 cattle ( $n = 15$  per system) were evaluated in three production systems: pasture, conventional feedlot, and feedlot for young beef bulls.

In the pasture, located in the municipality of Novo Progresso, in the state of Pará, Brazil, the production animals were fed a diet strictly of palisade grass [*Urochloa brizantha* (A.Rich.) R.D.Webster 'Marandu'] forage. Slaughter occurred when the animals were approximately 36 months old.

In the conventional feedlot, the animals were kept 84 days. The finishing diet was composed of 15% sugarcane bagasse in natura, 43% corn grain silage, 18.5% citrus pulp, 20% cotton seed cake, 2.5% mineral supplement, and 1.0% urea. The animals were slaughtered at an approximate age of  $24 \pm 6.86$  months.

In the young beef bull group, the experiment lasted 150 days at the experimental feedlot of UNESP, located in the municipality of Botucatu, in the state of São Paulo, Brazil. The animals were fed with a growing and finishing diet, containing 18 and 13% sugarcane bagasse in natura, 26.5% dry corn grain, 23% citrus pulp, 13% peanut bran, 15 and 20% rice bran, and 4.5% mineral supplement for young beef bull. The animals were slaughtered at 13 months.

To differentiate the feeding systems, 15 crossbred Nelore bulls were selected per system. Then, 150-g samples were collected from the ingredients of each feed and also from the *Trapezius cervicis*, *Longissimus dorsi*, and *Semitendinosus* muscles, in order to determine the incorporation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . The samples were stored under vacuum at  $-20^\circ\text{C}$  until isotopic measurement at the Center for Stable Isotopes of the Biosciences Institute of UNESP, also located in the municipality of Botucatu, in the state of São Paulo. In addition, samples from the ingredients of the production systems were analyzed and collected to assist in the interpretation of the results obtained for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Table 1).

For the isotopic analysis of carbon and nitrogen, the muscle samples were cut and dried in the MA035 forced-air oven (Marconi, Piracicaba, SP, Brazil), at  $50^\circ\text{C}$ , for 48 hours. Afterwards, portions of the samples were defatted with ethyl ether in a Soxhlet

apparatus, at 65°C, for 4 hours and then oven-dried, at 50°C, for 30 min to allow the evaporation of the ether. All muscle samples were ground in the 2010 Geno/Grinder cryogenic mill (Spex SamplePrep, Metuchen, NJ, USA), at -196°C. The samples were subsequently weighed, and 50–70 µg carbon and 500–600 µg nitrogen were put into 5x3-mm tin capsules and kept in the desiccator with P<sub>2</sub>O<sub>5</sub> for 24 hours prior to the analysis.

After weighing, the capsules were placed in the Flash 2000 elemental analyzer (Thermo Fisher Scientific, Bremen, Germany) for isotope ratio mass spectrometry. The gases formed during the process (CO<sub>2</sub> and NO<sub>x</sub>) were separated in a gas chromatographic column (3 m x 6 mm x 5 mm of inner diameter) and analyzed in the Delta V Advantage isotopic mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) at the Center for Stable Isotopes of the Biosciences Institute of UNESP. The carbon and nitrogen isotope data were reported in δ-notation in units of per thousands (‰) relative to the accepted international standards – Vienna Pee Dee Belemnite (VPDB) and atmospheric air, respectively –, being calculated as follows:

$$\delta \text{ (sample; reference)} = [(R_{\text{sample}}/R_{\text{reference}})-1] \times 1000$$

where δ is the relative enrichment of the sample in relation to the standard (dimensionless), and R is the isotopic ratio of the sample and standard (also dimensionless).

The reference working gases, CO<sub>2</sub> and N<sub>2</sub>, were calibrated with the urea isotopic working standard IVA33802174 (δ<sup>13</sup>C PDB = -39.73‰; δ<sup>15</sup>N air = 0.73‰). The analytical precision was 0.2‰ for both carbon and nitrogen.

The isotopic data of the δ<sup>13</sup>C and δ<sup>15</sup>N of the combination of non-defatted and defatted muscles were subjected to the multivariate analysis of variance, by the analysis of principal components (PCA), aiming to reduce the dimensionality of the data set and to

describe all of the variability of the system using a smaller number of variables. The discriminant analysis was also performed to create a profile of the muscles, used to distinguish and classify them, better discriminating between the production systems. The statistical software used was Minitab, version 16.0 (Minitab, LLC, State College, PA, USA).

## Results and Discussion

Based on the results of δ<sup>13</sup>C and δ<sup>15</sup>N obtained for the bovine muscles (Table 2), the PCA allowed the discrimination of the three production systems (Figure 1). For the *Trapezius cervicis* muscle, the first principal component explained 82.6% of the variability among the systems, and the highest contribution was that of carbon in the non-defatted muscles. The first two components explained 99.3% of total variation. For the *Longissimus dorsi* muscle, the first major component explained 88.5% of the observed variability, with a higher contribution of nitrogen in the defatted muscles. The first two components explained 98.7% of total variation. For the *Semitendinosus* muscle, the variability of the first principal component was 75.4%, and the highest contribution was of carbon in the defatted muscles. The first two components explained 98.5% of total variation.

For the three muscles, both non-defatted and defatted, the values obtained by the PCA explained 81.7% of the observed variability, and the highest contribution was of nitrogen in the defatted *Longissimus dorsi* muscle. The first two components explained 97.5% of total variation (Figure 1).

The proportions of isotopes δ<sup>13</sup>C and δ<sup>15</sup>N also allowed characterizing the three production systems evaluated. The samples from the young beef bull feedlot showed the highest δ<sup>13</sup>C values (-18.23±0.59‰ for non-defatted muscles and -17.75±0.40‰ for defatted ones), compared with those of the conventional feedlot system

**Table 1.** Enrichment of δ<sup>13</sup>C and δ<sup>15</sup>N in samples from diets (n=3) supplied in three different rearing systems during the production phases of beef cattle in Brazil.

Isotope	Conventional feedlot		Young beef bull feedlot			Pasture
	Breeding/Rearing	Finishing	Breeding	Rearing	Finishing	Breeding/Rearing/Finishing
δ <sup>13</sup> C (‰)	-14.24	-18.85	-13.70/-20.11	-22.18	-23.07	-14.41
δ <sup>15</sup> N (‰)	3.32	3.89	5.47/0.64	4.35	4.52	13.80

(-15.16±0.65‰ for non-defatted and -14.45±0.58‰ for defatted) and of the pasture (-12.59±0.49‰ for non-defatted and -12.24±0.46‰ for defatted). The highest  $\delta^{15}\text{N}$  was found in the pasture system (12.06±0.61‰ for non-defatted and 12.15±0.58‰ for defatted), followed by the young beef bull feedlot (7.24±0.37‰ for non-defatted and 7.37±0.44‰ for defatted) and the conventional feedlot (6.39±0.56‰ for non-defatted and 6.05±0.54‰ for defatted).

In the discriminant analysis, the isotopic values of the non-defatted and defatted muscle samples were used to establish a classification model, which was then evaluated by a cross-validation procedure. An adequate classification was reached with a correct global classification rate of 100% and a cross-validation rate of 100%. This classification model can be used to clearly discriminate the samples between the production systems, regardless of the evaluated muscles.

Considering the obtained results and the different metabolic rates of the studied muscle tissues, it was possible to differentiate the dietary composition of the three production systems through the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the non-defatted and defatted muscles. The proportions of the ingredients contained in each system influenced the isotopic values of carbon and nitrogen in the bovine muscles (Guo et al., 2010; Zhao et al., 2013). In the case of the pasture system, the animals were fed exclusively 'Marandu' palisade grass, characterized as a  $\text{C}_4$  forage, while, in the conventional feedlot and the young beef bull feedlot, the cattle were fed feeds with  $\text{C}_3$  and  $\text{C}_4$  plants (Table 2).

Lower  $\delta^{13}\text{C}$  values were found for the young beef bull feedlot. One possible explanation is that, before the intake of the diet, the cattle were creep fed during the rearing phase. Another is that the ingredients that make up this system are partly  $\text{C}_3$  plants, such as peanut meal, rice bran, citrus pulp, and soybean oil. De Smet et al. (2004), studying a feedlot of young beef bulls, found that the analysis of  $\delta^{13}\text{C}$  from tissues can be used as a method to track diets containing variable ratios of  $\text{C}_3$  and  $\text{C}_4$  plants.

The requirement of the young beef bulls for non-digestible protein in the rumen are higher (NRC, 1996) than that of the animals older than 18 months in the conventional feedlot system, increasing the use of  $\text{C}_3$  ingredients. In addition, the young bulls are kept longer in the feedlot system, favoring the exchange of the elements present in their diets, which are then incorporated into their tissues (Schwägele, 2005).

In a study testing the isotope technique in the traceability system for the authentication of animal products, Bahar et al. (2005) verified that the composition of the carbon and nitrogen stable isotopes in the bovine muscle changed considerably when grass silage ( $\text{C}_3$ ) was replaced by corn silage ( $\text{C}_4$ ) in the beef cattle diets. However, the muscle tissue evaluated was not suitable for short and recent changes in the diets, since, after 167 days of the experiment, tissue turnover had not been completed yet. This explains the results found in the present study, in which animals from the feedlot did not reach the isotope equilibrium level of the diet (Table 1).

**Table 2.** Values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in samples from non-defatted and defatted muscles of beef cattle in three rearing systems<sup>(1)</sup>.

Muscle	Conventional feedlot		Young beef bull feedlot		Pasture	
	Non-defatted	Defatted	Non-defatted	Defatted	Non-defatted	Defatted
<i>Trapezius cervicis</i>						
$\delta^{13}\text{C}$	-15.22±0.47	-14.45±0.59	-19.02±0.56	-17.86±0.25	-12.4±0.36	-12.01±0.32
$\delta^{15}\text{N}$	6.56±0.39	6.24±0.56	7.72±0.30	7.72±0.47	11.99±0.61	12.05±0.67
<i>Longissimus dorsi</i>						
$\delta^{13}\text{C}$	-15.73±0.63	-14.72±0.57	-18.00±0.44	-17.76±0.76	-13.19±0.55	-12.13±0.36
$\delta^{15}\text{N}$	6.91±0.29	6.32±0.35	6.87±0.25	7.13±0.37	12.70±0.53	12.68±0.60
<i>Semitendinosus</i>						
$\delta^{13}\text{C}$	-14.54±0.85	-13.75±0.45	-17.67±0.35	-17.62±0.25	-12.18±0.34	-12.57±0.93
$\delta^{15}\text{N}$	5.72±0.59	5.58±0.65	7.13±0.27	7.25±0.56	11.48±0.53	11.71±0.56

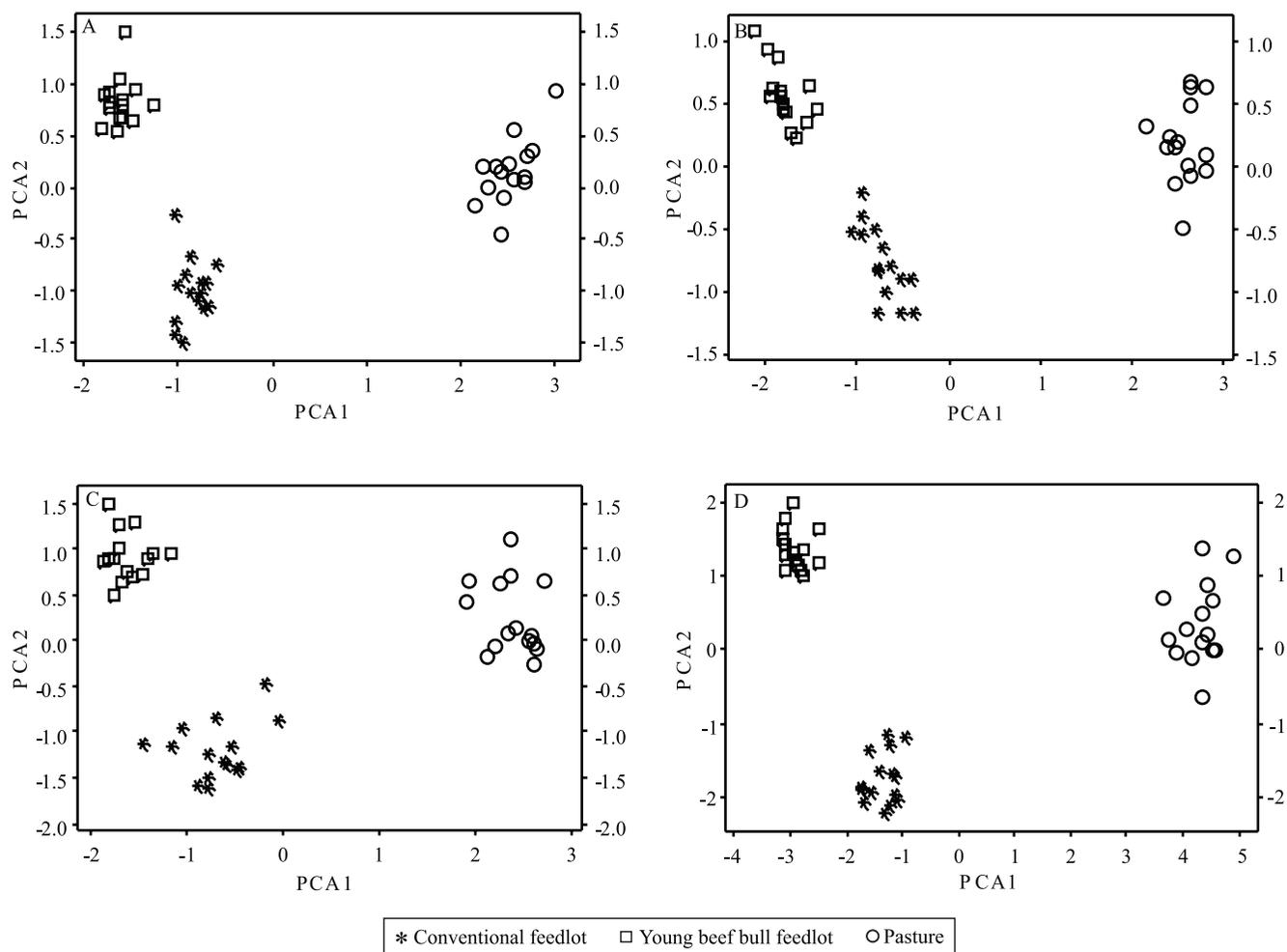
<sup>(1)</sup>Values are means ± standard deviation.

The  $\delta^{15}\text{N}$  values in the muscles of the animals reared in the pasture were the highest. This is interesting since this area did not receive any type of soil fertilization, meaning that the nitrogen absorbed by the forage came only from the organic matter decomposed on the soil. Some studies have shown that the nitrogen in animal muscles is characterized predominantly by the consumed diet and the local soil (Inácio & Chalk, 2017; Piasentier et al., 2003). Moreover,  $\delta^{15}\text{N}$  can reach values around 10‰ in soils with a high organic matter content (Shibuya et al., 2006), confirming the results obtained in this system in the present study.

In the conventional and young beef bull feedlots, the diets were composed mainly of grains, whose productivity is often increased through inorganic

fertilization. Several authors have reported a relative enrichment of about 0‰ for  $\delta^{15}\text{N}$  in different cultures due to this type of fertilization (Handley & Raven, 1992; Yoneyama, 1996; Werner & Schmidt, 2002). Furthermore, some legumes, such as soybean, also used in the feedlot diets in the present study, may lead to lower  $\delta^{15}\text{N}$  values (Zhao et al., 2013).

The assimilation of the elements of a diet by the different tissues of an animal is influenced by physiological, biochemical, and ecological factors (Gannes et al., 1997). In the present study, it was observed that metabolic factors inherent to the muscles also interfere in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the foods after animal intake, particularly the predominant type of muscle fibers. In the *Trapezius cervicis* muscle,



**Figure 1.** Principal component analysis of the following non-defatted and defatted muscles: *Trapezius cervicis* (A), *Longissimus dorsi* (B), and *Semitendinosus* (C), as well as the mean values of all muscles (D) in three production systems.

there was a prevalence of 62.6% oxidative fibers, while, in the *Longissimus dorsi* and *Semitendinosus* muscles, the glycolytic fibers represented the largest portions of 43.2 and 49.7%, respectively (Kirchofer et al., 2002). Oxidative fibers are closely linked to a high metabolism and oxygen-exchange dependency, being richly vascularized and having a large number of mitochondria (Macari et al., 1994), whereas glycolytic fibers show a reduced density of blood capillaries, with consequently small changes in the metabolites (Banks, 1992). Therefore, the greater the exchange of metabolites, the faster the muscles reflect the signal of the diet change, which can be observed in the total values of the coefficients of variability of the PCA for the *Trapezius cervicis* muscle. In addition, the type of production system adopted influences the physicochemical and qualitative characteristics of the muscles due to the level of physical activity, amount of concentrate in the diet, and, to a lesser extent, animal age (Therkildsen et al., 1998; Vestergaard et al., 2000).

When defatted, both the *Longissimus dorsi* and *Trapezius cervicis* muscles showed a greater enrichment in the assessed elements, i.e.,  $\delta^{13}\text{C} = 0.77\text{‰}$  and  $\delta^{15}\text{N} = 0.11\text{‰}$  and  $\delta^{13}\text{C} = 0.63\text{‰}$  and  $\delta^{15}\text{N} = 0.09\text{‰}$ , respectively; the *Semitendinosus* muscle was less affected, with  $\delta^{13}\text{C} = 0.15\text{‰}$  and  $\delta^{15}\text{N} = -0.07\text{‰}$ . This may be attributed to the increased presence of intramuscular fat in these muscles, which has a positive correlation with the type of fiber in the tissue (Hwang et al., 2010), since the *Longissimus dorsi* muscle has 10.7% more oxidative fibers than the *Semitendinosus* muscle. Moreover, the deposition of intramuscular fat occurs later in the carcass and is directly linked to the amount of energy in the diet (Silva et al., 2009), showing a better isotopic signature of the biological elements in the tissue.

### Conclusion

The carbon and nitrogen isotopic ratio of the *Trapezius cervicis*, *Longissimus dorsi*, and *Semitendinosus* non-defatted and defatted muscles allows for the precise identification of beef cattle production systems in tropical conditions.

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