# Effect of Seasons on Enteric Methane Emissions from Cattle Grazing Urochloa brizantha

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## Abstract

The objective of this study was to evaluate the effect of seasons under a tropical climate on forage quality, as well the effect of an *Urochloa brizantha* cv. Marandu grazing system on enteric methane (CH<sub>4</sub>) emissions from Nellore cattle in the Southeast region of Brazil. Sixteen Nellore steers (18 months old and initial weight 318.0  $\pm$  116.59 kg of LW; final weight 469  $\pm$  98.50 kg of LW) were used for a trial period of 10 months, with four collection periods in winter (August), spring (December), summer (February) and autumn (May). Each collection period consisted of 28 days, corresponding to the representative month of each season where the last six days were designed for methane data collection. Animals were randomly distributed within 16 experimental plots, distributed in four random blocks over four trial periods. CH<sub>4</sub> emissions were determined using the sulphur hexafluoride (SF<sub>6</sub>) tracer gas technique measured by gas chromatography and fluxes of CH<sub>4</sub> calculated. The forage quality was characterized by higher CP and IVDMD and lower lignin contents in spring, differing specially from winter forage. Average CH<sub>4</sub> emissions were between 102.49 and 220.91 g d<sup>-1</sup> (37.4 to 80.6 kg ani<sup>-1</sup> yr<sup>-1</sup>); 16.89 and 30.20 g kg<sup>-1</sup> DMI; 1.35 and 2.90 Mcal ani<sup>-1</sup> d<sup>-1</sup>; 0.18 and 0.57 g kg<sup>-1</sup> ADG<sup>-1</sup> and 5.05 and 8.76% of GE. Emissions in terms of CO<sub>2</sub> equivalents were between 4.68 and 14.22 g CO<sub>2</sub>-eq<sup>-1</sup> g<sup>-1</sup> ADG. Variations in CH<sub>4</sub> emissions were related to seasonal effect on the forage quality and variations in dry matter intake.

Keywords: CO<sub>2</sub>-eq, methane, Nellore, sulphur hexafluoride, Urochloa brizantha

## 1. Introduction

In Brazil, cattle represent 83.9% of all livestock production (of which 89% is beef cattle and 11% dairy cattle). Extensive production systems predominate and main national herd is composed by Zebu cattle (*B. indicus*), of which Nellore is the most numerous breed (80%), raised in predominantly extensive systems (Lima et al., 2010). The main food source is tropical forages, especially, the genus *Urochloa*, which occupy about 50% of the cultivated pastures area due to the low production cost compared to systems using confined animals and grains in the diet (Berchielli et al., 2012). As Brazil has the second largest cattle herd in the world (FAO, 2013) and the feed system is based on tropical forages, the country has been indicated as a methane-producing potential especially when the diet consists in low nutritional value forage (Canesin et al., 2014) which favours the low performance of animals and the increased production of greenhouse gases (GHGs), mainly enteric methane (CH<sub>4</sub>). However, these emissions could be reduced with cattle supplementation on pasture and proper management of grassland ecosystem which acts in favour of carbon sequestration (IPCC, 2007). The *Urochloa brizantha* cv. Marandu is a perennial forage grass of cespitose growth habit, forming clumps of up to 1.0 m in diameter and tillers with height of up to 1.5 m. It is well adapted and has good forage production in natural fertile soils; excellent performance in sandy soils; deep root system which allows to obtain water during dry periods; is more palatable than the other species of the genus, and therefore is widely used (Costa et al., 2004).

Cattle farming is a significant source of methane (CH<sub>4</sub>) gas emissions, an important contributing factor towards global warming (IPCC, 2007). Methane is produced as a result of the natural digestive process of ruminant herbivores. This process occurs in the rumen as result of a symbiotic relationship between ruminant and ruminal microbiota consisting of bacteria, protozoa and fungi. Fermentation that occurs during metabolism, especially of ingested carbohydrates as vegetative matter, is an anaerobic process carried out by ruminal microorganisms which convert cellulosic carbohydrates into short-chain fatty acids, mainly acetic, propionic and butyric acid (Lima et al., 2006). During the fermentative process heat is dissipated over the body surface and carbon dioxide (CO<sub>2</sub>) and CH<sub>4</sub> gas are expelled into the atmosphere via eructation and respiration to avoid the prejudicial effect of excessive hydrogen (H<sub>2</sub>) production to animal health (Dukes et al., 1977). Methane emission represent a loss of between 5% and 8% of gross energy intake according to Blaxter and Clapperton (1965), and between 2% and 12% according to K. A. Johnson and D. E. Johnson (1995), while IPCC provides estimates of 3 to 6.5% (IPCC, 2006). Considering that CH<sub>4</sub> production varies in accordance to the physiological state and type of animal (Lima et al., 2006) as well as, with the quantity and quality of ingested food (USEPA, 1990a, 1990b), various cattle production systems will result in different factors of methane emission.

Global CH<sub>4</sub> emission from enteric fermentation by ruminants are estimated to be in the range of 76 to 92 million tonnes per year (Dlugokenky et al., 2011), approximately 16% of the total from anthropogenic sources, while those originating from animal manures are estimated at 25 million tons of CH<sub>4</sub> per year (Mosier et al., 2004), approximately 5% of the total from anthropogenic sources. Methane emissions from livestock production in Brazil were estimated at around 11.5 million tonnes per year (Lima et al., 2010), taking into account ruminal and manure emission in 2005. Beef and dairy cattle alone account for 97% of enteric CH<sub>4</sub> emissions from agricultural sources in the country, with the other 3.0% attributable to other categories of animals (buffalo, mules, goats, asses, horses, swine) (Lima et al., 2010). This scenario demonstrates the importance of studies that provide data on the real contribution of ruminants under tropical conditions to greenhouse gas emissions as well as providing indications for reducing CH<sub>4</sub> emissions from livestock production via strategic nutritional and feed management (Tamminga, 1992; Holter & Young, 1992).

Bearing in mind this scenario, the objective of this study was to evaluate the effect of seasons on forage quality and consequently enteric  $CH_4$  emission under the climatic conditions of the Southeast region of Brazil over an one-year period, using the  $SF_6$  technique with Nellore cattle grazing *Urochloa brizantha* cv. Marandu pasture.

### 2. Materials and Methods

### 2.1 Location and Experimental Area

The experiment was conducted at the Institute of Animal Science and Pastures (IZ) that belongs to the São Paulo State Department of Agriculture and Food Supply (SAA) and interacts through the São Paulo Agency of Agribusiness and Technology (APTA). IZ is located in the municipality of Nova Odessa at an average altitude of 560 m with geographical coordinates of 22°42′S latitude and 47°18′W longitude, with soil characterized as dark red yellow latosol (Oxisol). Tropical climate predominates with a mean annual rainfall of 1367 mm and average annual temperature of 21.7 °C. The dry season is characterized by a cold period comprising autumn season (March to May, with a minimum average temperature of 15.5 °C and a maximum average of 28.0 °C and average rainfall of 102.86 mm) and winter season (June to August, with a minimum average temperature of 11.4 °C and maximum average of 25.8 °C and average rainfall of 27.86 mm). While the wet season is characterized by a hot period covering spring season (September to November, with a minimum average temperature of 15.6 °C and a maximum average of 28.8 °C and average rainfall of 109.9 mm) and summer (December to February, with a minimum average temperature of 18.8 °C and average maximum of 30.0 °C and average rainfall of 215.3 mm) (CEPAGRI, 2015).

Evaluation was carried out in an area of 48 hectares, divided into 48 paddocks of 1 ha each. The experimental plot was represented by an area of 3 ha, composed of three paddocks totalling therefore 16 experimental plots. All experimental plots were equally managed so that seasonal effects should remain homogenous across the 16 plots. Grazing was rotated within the experimental area with rest and occupation periods of 56 and 28 days respectively during the dry season and of 42 and 21 days during the wet season. Pasture used for the trial consisted of the species *Urochloa brizantha* (syn. *Brachiaria brizantha*) cultivar Marandu (seeded 12 years ego). The only feed supplement daily provided and ad libitum was a complete mineral mix specifically for beef cattle in the stocker or backgrounding phase, with no added protein and energy content.

### 2.2 Animals and Experimental Design

Sixteen Nellore steers of 18 months old (initial weight  $318.0 \pm 116.59$  kg of live-weight (LW); final weight 469  $\pm$  98.50 kg of LW) were used for a trial period of 10 months from July to May with four collection periods,

represented by each season, winter (August), spring (December), summer (February) and autumn (May). Each collection period consisted of 28 days, corresponding to the representative month of each season where the last six days were designed for methane data collection. Animals were weighed at the beginning and at the end of each trial period and remained at grazing rotational grazing during the whole experimental period. The animals were randomly assigned to each experimental plot, along with other regulatory animals which were required to maintain adequate management of the pasture.

An experimental design of randomized blocks was used, represented by 16 animals, each one in a separate paddock, totalling 16 trial plots distributed in four blocks over four trial periods, applied in an *Urochloa brizantha* cultivar Marandu pasture grazing system. In this manner the model takes into account block effects (4) and treatment effects (seasons of the year).

# 2.3 Evaluation of Availability and Quality Analysis of Forage

Forage availability was estimated using the square method (Gardner, 1967) on the first day of data collection. Forage samples were taken at randomly allocated points within the trial areas before animals were released into the plot and shortly after their removal by cutting manually with garden shears at a height of approx. 5 cm above the ground. Material was collected by paddock and by season before being dried in a forced air heater at 65 °C for 72 hours. Samples were ground through a 1.5 mm screen for later determination of dry matter content (DM, Method 934.01; AOAC, 1990); mineral content (MM, Method 923.03; AOAC, 1990); crude protein content (CP, Method 920.87; AOAC, 1990); ether extract content (EE, determined gravimetrically after extraction using ethyl ether in a Soxhlet extractor - Method 920,85; AOAC, 1990); and neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and lignin content following Van Soest (1994). Nutritional analyses were conducted at the Animal Nutrition Laboratory of the Institute of Animal Science and Pastures (IZ), Nova Odessa, SP (Brazil).

## 2.4 Dry Matter Intake

Dry matter intake (DMI) of forage and of total digestible nutrients (TDN) was estimated for each animal using the Cornell Net Carbohydrate and Protein System (5.0) program. *In vitro* dry matter digestibility (IVDMD) was considered to be equal to TDN, and consequently it was considered that 1kg of dry matter consumed was equivalent to 4.44 Mcal of digestible energy (DE) following NRC (1996).

## 2.5 CH<sub>4</sub> Measurement

The internal tracer sulphur hexafluoride (SF<sub>6</sub>) technique was employed for the measurement of  $CH_4$ , as described by K. A. Johnson and D. E. Johnson (1995) and adapted in Brazil by Primavesi et al. (2004b).

Five days prior to the start of the first gas collection with collection canisters (container in the form of a yoke fabricated from 60 mm class 20 PVC tubing with an internal pressure of close to zero atmospheres), permeation tubes loaded with  $SF_6$  (566.7 mg), calibrated and identified, with known constant release rates (2.06 ± 0.71 mg of  $SF_6 d^{-1}$ ), were introduced into the rumen of the animals to remain until completion of the final trial period. The  $CH_4$  sampling was conducted during winter (August), spring (December), summer (February) and autumn (May) in animals equipped with air-sampling apparatus consisting of a halter (with stainless steel capillary tube) and collection canister (in the form of a yoke) coupled to a metal shut-off valve and quick-connect. A high vacuum pump was employed for this technique with two stages and a digital manometer with a range of 0 to 203 kPa (2 atm. or 29.4 psi or 1,520 mm Hg; on the scale of 0 to 2 atm), which permitted the measurement of the initial and final pressure of each canister during the data collection period.

Before use, the halters were calibrated so as to reach half an atmosphere of pressure after 24 hours of gas collection by using 0.127 mm internal diameter stainless steel capillary tube attached to the halter. The calibration was determined by the length of the capillary tubing.

After the animals had adapted to the presence of the collection canisters (during days 18-22 of each trial period), measurements of CH<sub>4</sub> production using the tracer gas SF<sub>6</sub> were carried out at 24-hour intervals for six days from the 23rd day of grazing. Concentrations of CH<sub>4</sub> and SF<sub>6</sub> were determined by gas chromatography at EMBRAPA Meio Ambiente, in Jaguariúna, São Paulo State (Brazil), using a HP6890 gas chromatograph (Agilent, Delaware, USA), equipped with Flame ionization detector (FID) to 280 °C, megabore column (0.53 mm × 30 m × 15 µm) Plot HP-Al/M (for CH<sub>4</sub>), electron capture detector (ECD) to 300 °C and megabore column (0.53 mm × 30 m × 25 µm) HP-MolSiv (for SF<sub>6</sub>), with two 0.5 cm<sup>3</sup> loops coupled to two 6-port valves. The gas chromatography oven was maintained at 50 °C during the analysis. Directly after the sample collection period and before the determination of CH<sub>4</sub> and SF<sub>6</sub> concentrations, the collection canisters were pressurized with nitrogen 5.0 to pressures of 1.3 to 1.5 psi (g), with initial and final dilution readings taken using a portable digital manometer (±0.01), certified for a reading scale of -1 to +2 bar (g) (Druck, model DPI705), to obtain the dilution factor. The

calibration curves were established based on certified gas standards (White Martins), with ppm concentrations for CH<sub>4</sub> (4.85 and 20 ppm) and ppt for SF<sub>6</sub> ( $34 \pm 9$ ,  $91 \pm 9$  and  $978 \pm 98$  ppt), as related by Johnson et al. (2007).

Emission rate of CH<sub>4</sub> was calculated based on the known release rate of the tracer in the rumen and concentrations of methane and the tracer in samples from the environment and in the gas samples collected from the animals. Later, the primary data obtained was used to calculate the emission potential in grams of CH<sub>4</sub> per day – (CH<sub>4</sub>, g d<sup>-1</sup>); grams of CH<sub>4</sub> per kilogram of dry matter intake – (CH<sub>4</sub>, g kg<sup>-1</sup> DMI); grams of CH<sub>4</sub> per kilogram of digestible dry matter – (CH<sub>4</sub>, g kg<sup>-1</sup> DDM); megacalories of digestible energy per animal per day – (DEI, Mcal ani<sup>-1</sup> d<sup>-1</sup>); megacalories of CH<sub>4</sub> per animal per day – (CH<sub>4</sub>, g hg<sup>-1</sup> DMI); grams of CH<sub>4</sub> per animal per day – (CH<sub>4</sub>; percentage of gross energy lost in the form of methane – CH<sub>4</sub> (%GE) considering 13.16 Mcal g<sup>-1</sup> of CH<sub>4</sub>; percentage of gross energy lost in the form of methane – CH<sub>4</sub> (%GE) considering 4.4 Mcal of energy per kg of dry matter (Holter & Young, 1992); percentage of digestible energy lost in the form of methane – CH<sub>4</sub> (%DE), with digestible energy estimated from the digestive percentage of crude energy; grams of CH<sub>4</sub> per kilogram of average daily gain – (CH<sub>4</sub>, g kg<sup>-1</sup> ADG) and emission intensity in CO<sub>2</sub> equivalents per kilogram of average CH<sub>4</sub> emissions for the season in which the data was collected.

## 2.6 Statistical Analysis

Results for forage availability and quality as *in vitro* dry matter digestibility, intake of dry matter (DMI), crude protein (CPI) and neutral detergent fibre (NDFI), as well rates of enteric emissions of  $CH_4$  were submitted to analysis of variance and its effects were evaluated by the Tukey test (P < 0.05) using the Statistical Analysis System program (Version 9.2, 2010). The SAS's MIXED procedure was used for these variables. The model considered the effect of the season and the block effect as fixed and random variables respectively.

### 3. Results

The season of the year was found to have a significant effect on forage availability (P < 0.05) measured in kilograms of dry matter per hectare (kg DM ha<sup>-1</sup>) and on each of the variables representing its chemical composition (Table 1).

Forage availability was confirmed to be greater in winter than spring and summer but not different from autumn (P < 0.05). This greater availability was probably due to the management strategy of deferment (leaving pasture unoccupied to grow before the dry winter season) which the pasture had been subjected to before the trial periods began. However, the forage itself contained higher levels (P < 0.05) of DM, ADF and Lignin than the other seasons. Likewise, NDF content was higher in both winter and autumn than in summer and spring (the last two also differing from each other). CP, MM and EE content and *in vitro* dry matter digestibility were lower (P < 0.05) in winter compared with the other seasonal trial periods.

Variables <sup>1</sup>		Season				Probability <sup>2</sup>
	Winter	Spring	Summer	Autumn	– SEM	Flobability
DM (kg ha <sup>-1</sup> )	6412.07 <sup>a</sup>	3670.41 <sup>c</sup>	4777.64 <sup>bc</sup>	5538.86 <sup>ab</sup>	232.57	< 0.0001
DM (%)	61.86 <sup>a</sup>	25.98 <sup>c</sup>	24.18 <sup>c</sup>	29.88 <sup>b</sup>	1.99	< 0.0001
MM (% DM)	6.20 <sup>b</sup>	8.12 <sup>a</sup>	8.17 <sup>a</sup>	8.50 <sup>a</sup>	0.13	< 0.0001
CP (% DM)	3.33 <sup>c</sup>	7.72 <sup>a</sup>	5.35 <sup>b</sup>	5.56 <sup>b</sup>	0.21	< 0.0001
EE (% DM)	0.63 <sup>c</sup>	1.64 <sup>a</sup>	1.08 <sup>b</sup>	1.14 <sup>b</sup>	0.05	< 0.0001
NDF (% DM)	81.73 <sup>a</sup>	70.83 <sup>c</sup>	77.17 <sup>b</sup>	82.48 <sup>a</sup>	0.60	< 0.0001
ADF (% DM)	51.37 <sup>a</sup>	41.22 <sup>d</sup>	44.07 <sup>c</sup>	48.62 <sup>b</sup>	0.52	< 0.0001
Lignin (% DM)	7.79 <sup>a</sup>	4.35 <sup>c</sup>	4.69 <sup>c</sup>	5.93 <sup>b</sup>	0.18	< 0.0001
IVDMD	41.38 <sup>c</sup>	60.38 <sup>a</sup>	62.45 <sup>a</sup>	55.98 <sup>b</sup>	1.13	< 0.0001

Table 1. Effect of the seasons of the year on availability and quality of *Urochloa brizantha* cv. Marandu pasture, expressed as percentage of dry matter (% DM)

*Note.* <sup>1</sup>DM = dry matter, MM = mineral content, CP = crude protein, EE = ether extract, NDF = neutral-detergent fibre, ADF = acid-detergent fibre, IVDMD = *In vitro* dry matter digestibility; <sup>2 a,b</sup> Different letters in the same line differ significantly (P < 0.05) using the Tukey test (PDIFF).

As for dry matter intake, expressed in kilograms per day (kg  $d^{-1}$ ) or as a percentage of live weight (% LW); crude protein intake – (CPI kg  $d^{-1}$ ); neutral-detergent fibre intake – (NDFI kg  $d^{-1}$ ) and kilograms of digestible dry

matter (DDM kg<sup>-1</sup>), as presented in Table 2, there were significant differences (P < 0.05) in terms of seasonal effects for each of these analysed variables. This occurred as a result of the chemical variation in the forage material which was the primary cause of differences in dry matter intake and consequently protein and neutral-detergent intake.

Table 2. Effect of season on dry matter, crude protein and neutral-detergent fibre intake in Nellore cattle grazing *Urochloa brizantha* cv. Marandu

Variables <sup>1</sup>		Season				Probability <sup>2</sup>
	Winter	Spring	Summer	Autumn	— SEM	Tiobability
DMI (kg $d^{-1}$ )	6.08 <sup>b</sup>	7.76 <sup>a</sup>	7.26 <sup>ab</sup>	7.58 <sup>ab</sup>	0.20	0.0188
DMI (% LW)	2.00 <sup>b</sup>	2.41 <sup>a</sup>	1.81 <sup>bc</sup>	1.73 <sup>c</sup>	0.04	< 0.0001
CPI (kg d <sup>-1</sup> )	0.20 <sup>c</sup>	0.55 <sup>a</sup>	0.39 <sup>b</sup>	0.42 <sup>b</sup>	0.02	< 0.0001
NDFI (kg d <sup>-1</sup> )	5.00 <sup>b</sup>	5.54 <sup>ab</sup>	5.60 <sup>ab</sup>	6.26 <sup>a</sup>	0.16	0.0184
DDM (kg <sup>-1</sup> )	2.54 <sup>b</sup>	4.70 <sup>a</sup>	4.53 <sup>a</sup>	4.24 <sup>a</sup>	0.15	< 0.0001

*Note.* <sup>1</sup>DMI = dry matter intake, expressed in kilograms per day or as a percentage of live weight, CPI = crude protein intake, NDFI = neutral-detergent fibre intake, DDM = digestible dry matter; <sup>2 a,b</sup> Different letters in the same line differ significantly (P < 0.05) using the Tukey test (PDIFF).

Significant differences in  $CH_4$  emissions were observed among seasons (Table 3). Emissions were highest during summer compared to the other seasons for all evaluated variables, except for  $CH_4$  emissions expressed per kilogram of average daily gain. Summer differed from the other seasons (P < 0.05) in terms of  $CH_4$  emission in grams per day – ( $CH_4$ , g d<sup>-1</sup>), with spring not differing from winter and autumn and with the lowest emissions recorded in winter.

Variables <sup>1</sup>		Season				Drohohilita <sup>2</sup>
	Winter	Spring	Summer	Autumn	– SEM	Probability <sup>2</sup>
Weight (kg)	317.56 <sup>b</sup>	332.75 <sup>ab</sup>	410.75 <sup>ab</sup>	448.00 <sup>a</sup>	16.05	0.0102
MW ( $kg^{0.75}$ )	74.35 <sup>b</sup>	77.04 <sup>b</sup>	90.58 <sup>ab</sup>	96.86 <sup>a</sup>	2.71	0.0079
ADG (g $d^{-1}$ )	335.0 <sup>bc</sup>	213.5 <sup>c</sup>	1258.1ª	497.2 <sup>b</sup>	3.88	< 0.0001
$CH_4 (g d^{-1})$	102.49 <sup>c</sup>	132.00 <sup>bc</sup>	220.91 <sup>a</sup>	159.98 <sup>b</sup>	8.02	< 0.0001
CH <sub>4</sub> (g kg <sup>-1</sup> DMI)	17.13 <sup>cd</sup>	16.89 <sup>d</sup>	30.20 <sup>a</sup>	21.65 <sup>b</sup>	0.87	< 0.0001
CH <sub>4</sub> (g kg <sup>-1</sup> DDM)	41.59 <sup>ab</sup>	28.04 <sup>c</sup>	48.55 <sup>a</sup>	38.77 <sup>b</sup>	1.38	< 0.0001
DEI (Mcal ani <sup>-1</sup> d <sup>-1</sup> )	11.27 <sup>b</sup>	20.87 <sup>a</sup>	20.13 <sup>a</sup>	18.81 <sup>a</sup>	0.70	< 0.0001
$CH_4$ (Mcal ani <sup>-1</sup> d <sup>-1</sup> )	1.35 <sup>c</sup>	1.74 <sup>bc</sup>	2.90 <sup>a</sup>	2.10 <sup>b</sup>	0.10	< 0.0001
CH <sub>4</sub> (% GE)	5.12 <sup>c</sup>	5.05 <sup>c</sup>	8.76 <sup>a</sup>	6.18 <sup>b</sup>	0.23	< 0.0001
CH <sub>4</sub> (% DE)	12.33 <sup>ab</sup>	8.31 <sup>c</sup>	14.39 <sup>a</sup>	11.49 <sup>b</sup>	0.41	< 0.0001
CH <sub>4</sub> (g kg <sup>-1</sup> ADG)	0.37 <sup>ab</sup>	0.57 <sup>a</sup>	0.18 <sup>b</sup>	0.42 <sup>a</sup>	0.03	0.0003
$CH_4$ (g $CO_2$ -eq <sup>-1</sup> g <sup>-1</sup> ADG)	9.32 <sup>ab</sup>	14.22 <sup>a</sup>	4.68 <sup>b</sup>	10.71 <sup>a</sup>	0.87	0.0003

Table 3. Effect of the seasons on enteric methane emissions from Nellore cattle grazing *Urochloa brizantha* cv. Marandu

*Note.* <sup>1</sup>MW = metabolic weight, ADG = average daily gain, CH<sub>4</sub>, g d<sup>-1</sup> = emission potential in grams of CH<sub>4</sub> per day, CH<sub>4</sub>, g kg<sup>-1</sup> DMI = grams of CH<sub>4</sub> per kilogram of dry matter intake, CH<sub>4</sub>, g kg<sup>-1</sup> DDM = grams of CH<sub>4</sub> per kilogram of digestible dry matter, DEI, Mcal ani<sup>-1</sup> d<sup>-1</sup> = megacalories of digestible energy per animal per day, CH<sub>4</sub>, Mcal ani<sup>-1</sup> d<sup>-1</sup> = megacalories of CH<sub>4</sub> per animal per day, CH<sub>4</sub>, Mcal ani<sup>-1</sup> d<sup>-1</sup> = megacalories of CH<sub>4</sub> per animal per day, CH<sub>4</sub>, Mcal ani<sup>-1</sup> d<sup>-1</sup> = megacalories of CH<sub>4</sub> per animal per day, CH<sub>4</sub>, Mcal ani<sup>-1</sup> d<sup>-1</sup> = megacalories of CH<sub>4</sub> per animal per day, CH<sub>4</sub> (%GE) = percentage of gross energy lost in the form of methane, CH<sub>4</sub> (%DE) = percentage of digestible energy lost in the form of methane, CH<sub>4</sub>, g kg<sup>-1</sup> ADG = grams of CH<sub>4</sub> per kilogram of average daily gain, g CO<sub>2</sub>-eq<sup>-1</sup> g<sup>-1</sup> ADG = emission intensity in CO<sub>2</sub> equivalents per kilogram of average daily gain. <sup>2 a,b</sup> Different letters in the same line differ significantly (P < 0.05) using the Tukey test (PDIFF).

## 4. Discussion

## 4.1 Availability and Quality of Forage

During the wet season (spring and summer) the forage was found to present lower DM, NDF, ADF and lignin content and higher CP content as well as increased IVDMD when compared to winter forage. Euclides et al. (2009) recorded similar results when evaluating the nutritive value of forage in *Urochloa brizantha* pasture throughout the year. These authors suggested that when FDN and acid-detergent lignin (ADL) content is lower the CP content of the forage and *in-vitro* digestibility of organic matter (IVOMD) is higher and vice-versa, irrespective of the cultivar analysed. Likewise, the authors cite that, independent of the experimental year of the forage quality analysis, CP content is higher and NDF content lower during the wet season than during the dry season. As such, variation in the nutritive value of *U. brizantha* cultivars during the year was a consequence of the climatic variations which occurred (Euclides et al., 2008, 2009), as well as the different flowering times of these cultivars (Valle et al., 2004) as these factors are determinants of the potential nutritive value of the forage source.

### 4.2 Nutrient Intake

Dry matter intake in kilograms per day was greater during spring than winter (P < 0.05) with these seasons not differing from summer and autumn for this variable. When dry matter intake was expressed in relation to live weight, once again there was an increase in spring, differing in turn from the other seasons with the lowest intake observed in the autumn.

The DM intake value as a percentage of live weight (% LW) of the grazing animals was close to the 2.5% value recommended by the NRC (1996) for beef cattle during the spring period, but values for winter (2.0%), summer (1.81%) and autumn (1.73%) were considered to be low. This could be attributed to a crude protein deficiency (Table 1) in the forage during these seasons, with CP contents lower than 7%, a percentage which guarantees adequate microorganism activity in the rumen (Van Soest, 1994).

A seasonal effect was observed (P < 0.05) for CPI and NDFI, with both being at their lowest level during winter, while CPI was highest during spring. Likewise, an increase in NDFI was observed during autumn in relation to winter, with these seasons not differing from summer and spring. This effect can be related to the forage chemical composition (Table 1) during each season and consequently to the obtained DMI value.

During spring, summer and autumn greater mass of digestible dry matter was observed (P < 0.05) than in winter. This was to be expected as the winter forage presented higher lignin content (Table 1), and was characterized as lower quality when compared to forage from other seasons. This resulted in a reduction in DMI and consequently in CPI and NDFI, probably due to a lower passage rate and greater retention time in rumen reflected as lower *in-vitro* dry matter digestibility.

## 4.3 Methane Emissions

The low  $CH_4$  emission rate obtained during winter is related to a DM consumption decrease during this season due to an inferior forage when compared to the other seasons (Kurihara et al., 1999). The forage is characterized as inferior mainly because of its higher fibre content and lower digestibility. This shows a direct relationship among forage quality, DM intake and consequently  $CH_4$  emissions. Various studies mention that the quantity of consumed feed is an important determinant of daily  $CH_4$  emissions in cattle and as such it has been included in all indicators of daily  $CH_4$  production (Blaxter & Clapperton, 1965; Benchaar et al., 1998).

Kurihara et al. (1999), found that Brahman heifers, consuming low quality tropical grasses (Angleton grass, *Dichanthium aristatum*), showed lower DM consumption (3.58 kg of DM d<sup>-1</sup>) and lower CH<sub>4</sub> emissions (113 g d<sup>-1</sup>). However, when animals had access to a higher quality forage source (Rhodes grass, *Chloris gayana*), consumption increased (7.07 kg of DM d<sup>-1</sup>) and consequently so did CH<sub>4</sub> emission (235 g d<sup>-1</sup>). A study by Nascimento et al (2016), evaluating CH<sub>4</sub> emission from Nellore cattle feeding on *Urochloa brizantha* hay harvested at different stages of maturity observed CH<sub>4</sub> emission values of between 132.7 and 138.3 g d<sup>-1</sup> for the different treatments, values close to those observed in this study.

For CH<sub>4</sub> emissions expressed in g kg<sup>-1</sup> DMI, values ranged from 16.9 to 30.3 g kg<sup>-1</sup> DMI, with highest emissions during summer (P < 0.05) and lowest emission during spring in relation to the other seasons. Similar values were observed by Possenti et al. (2008), when studying the effects of two different percentages of *Leucaena* hay content in the diet (20 and 50% DM), obtaining emissions of 20.5 and 16.9 g kg<sup>-1</sup> DMI, respectively. Likewise, Nascimento et al. (2016) cited CH<sub>4</sub> values of between 17.4 and 23.4 g kg<sup>-1</sup> DMI using *Urochloa brizantha* harvested at different stages of maturity.

The same seasonal effect (P < 0.05), observed in relation to  $CH_4$  emissions in g kg<sup>-1</sup> DMI, was also found for emissions expressed in g kg<sup>-1</sup> DDM, showing, once again, an increase during summer and lower emissions in spring. Values of between 28.0 and 48.6 g  $CH_4$  kg<sup>-1</sup> DDM were recorded, close to those obtained by Primavesi et al. (2004a) of between 42 and 69 g  $CH_4$  kg<sup>-1</sup> DDM using grazing dairy cattle Holstein of 600 kg LW. The findings for these last two variables may be attributable to the low fibre (NDF and ADF) content during the spring, these being main causes for lower  $CH_4$  emissions in g kg<sup>-1</sup> DDM as Kurihara et al. (1999) related, explaining that the highest values during summer are due to the highly digestible fibre components. These results allow us to state that a strong correlation exists between forage quality and  $CH_4$  production. Some authors mention that pasture improvement programs reduce  $CH_4$  production from grazing cattle by up to 10%, which seems possible when fibre content is reduced and digestible energy and crude protein content in the diet is increased (Kurihara et al., 1999).

 $CH_4$  emissions in Mcal ani<sup>-1</sup> d<sup>-1</sup>, were highest (P < 0.05) during summer compared to the other seasons, and were at their lowest during winter. This result was directly related to  $CH_4$  emissions in g d<sup>-1</sup> and dry matter consumption, demonstrating the same seasonal effect, with these variables at their lowest during winter. Once again, this effect is a result of forage quality in this season, with the winter pasture presenting greater fibre content (NDF and ADF) with lower digestibility (higher lignin content) and lower ether extract content (Table 1). It is important to consider that even with lower quality forage, such as that available during the winter period when compared with other seasons,  $CH_4$  emissions were lower both in g d<sup>-1</sup> and Mcal ani<sup>-1</sup> d<sup>-1</sup>. In low quality forage, this is because of that the addition of nutrients to the microorganisms increases the microbial growth efficiency by increasing the fermentation process efficiency in rumen, resulting in a decrease in methanogenic *Archaea* per unit of carbohydrate degraded (Cottle et al., 2011).

Percentage of CH<sub>4</sub> produced in relation to gross energy intake by cattle was higher (P < 0.05) during summer (8.76%) than autumn (6.18%), spring (5.05%) and winter (5.12%) with the last two seasons not differing significantly from each other and resulting in lower energetic losses as CH<sub>4</sub>. Values similar to those presented in this study were proposed by USEPA (2000) for North America and Eastern Europe (5.5-6.5% respectively), and also by Nascimento et al. (2016) for animals fed *Urochloa brizantha* hay. IPCC (2006) estimates losses of 6.5% to 3.0% for animals eating low quality tropical grasses and diets containing grains, respectively. The value for summer obtained by this study was greater than that reported by USEPA (2000) and IPCC (2006), but lower than found by Kurihara et al. (1999) studying animals fed low quality (10.4%) and high quality (11.4%) grasses.

Losses of digestible energy as CH<sub>4</sub> were greatest (P < 0.05) in summer (14.39%) in relation to autumn (11.49%) and spring (8.31%) which had the lowest losses but did not differ significantly from winter (12.33%). The lower energetic losses during spring are associated, as with other variables of CH<sub>4</sub> obtained, with the available forage quality during this season, with its lower fibre content and increased digestibility, as well as higher energetic content (%EE), highlighting the characteristic of ether extract as a more energetic fraction, which may have resulted in an increase in forage's utilizable energy.

Although  $CH_4$  emissions expressed by the different variables mentioned were higher during summer and lower during spring and winter, when emissions are expressed per unit of product (ADG), the seasonal effect is inversely proportional, with grams of  $CH_4$  emitted per kilogram of average daily gain being lowest (P < 0.05) in summer and highest in spring. This may have occurred as a result of the summer forage being more digestible than that of the other seasons, which could in turn have led to an increase in consumption and improved performance and consequently to a considerable reduction in  $CH_4$  emissions per kilo of weight gain.

Providing diets which contain large quantities of rapidly digestible carbohydrates or using higher quality forage results in greater productive performance. Moss and Givens (2002) cite that improved animal performance can reduce  $CH_4$  emissions due to the reduction in the number of animals in the production system, considering that in cattle reared for meat the increase in animal performance results in a shorter time within the system, reducing in this way the total  $CH_4$  produced during the life cycle.

Values for the intensity of CH<sub>4</sub> emissions converted into CO<sub>2</sub> equivalents in relation to average daily gain (g CO<sub>2</sub>-eq<sup>-1</sup> g<sup>-1</sup> ADG) were higher (P < 0.05) during spring and autumn in relation to summer and not significantly different from emission intensities observed during winter.

Available data referring to the intensity of  $CH_4$  emissions in equivalent  $CO_2$  emissions of 0.31 g  $CO_2$ -eq<sup>-1</sup>kg of milk d<sup>-1</sup> for dairy cows with an average of 34 kg of milk d<sup>-1</sup> is suggested by Ulyatt et al. (2002), and for dairies in developed countries are estimated at 1.0 to 1.6 kilos of  $CO_2$ -eq<sup>-1</sup>kg of milk by Pereira et al. (2011). Differences in emission intensities are attributed to management differences of production systems, including dietary composition and the cow's productivity levels. It is important to point out that there is little data available

referring to the intensity of  $CH_4$  emissions in  $CO_2$  equivalents per gram of average daily gain (g  $CO_2$ -eq<sup>-1</sup> g<sup>-1</sup> ADG) for beef cattle, highlighting the importance of continuing with studies determining emission intensities throughout the productive cycle, in different seasons, to supply accurate and precise information on the interaction between animal performance and greenhouse gas emissions.

#### 5. Conclusions

The results indicate differences in  $CH_4$  emissions resulting from variations in forage quality, dry matter consumption and seasons. Thereby, the high quality forage plants have lower fibre content and higher digestibility, resulting in increased daily dry matter intake, increased weight gain and consequently lower methane emission (g  $CO_2$ -eq<sup>-1</sup> g<sup>-1</sup> ADG).

Future experimentation should try to account for the large number of variables involved, considering enteric methane emissions per kilo of product (meat) throughout the animal's productive cycle, whilst also taking into account the importance of measuring emissions of methane and nitrous oxide from the soil as well as carbon sequestration, which could positively compensate for methane emissions.

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