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Nutritive value and in vitro methane production of *Urochloa brizantha* cv. Marandu under fixed time or variable stocking cycles

André Morais Moura[1] | Thierry Ribeiro Tomich[2] | Luiz Gustavo Ribeiro Pereira[2] | Domingos Sávio Campos Paciullo[2] | Carlos Augusto de Miranda Gomide[2] | Lucio Carlos Gonçalves[1]

[1]Department of Animal Science, Veterinary School, Federal University of Minas Gerais, Belo Horizonte, Brazil

[2]The Brazilian Agricultural Research Corporation/Embrapa (Dairy Cattle), Juiz de Fora, Brazil

Correspondence
André Morais Moura, Department of Animal Science, Veterinary School, Federal University of Minas Gerais, Av. Antônio Carlos, 6627, PO Box 567, Pampulha Campus, UFMG –ZIP CODE: 31270-901. Belo Horizonte, Minas Gerais, Brazil.
Email: amoraismoura@yahoo.com.br

Abstract

The aim of this study was to evaluate the chemical composition, digestibility, gas production kinetics and in vitro methane (CH_4) production of Urochloa brizantha cv. Marandu under two stocking methods over three consecutive stocking cycles. The stocking methods were (1) a fixed-time stocking cycle of 33 days (33-SC) with a 30-day rest period and a 3-day grazing period, and (2) a variable stocking cycle, with the end of the rest period when the canopy intercepted 95% of the light and a 3-day grazing period (95-LI). The average rest period was 24.5-day (standard deviation of 5.2-day) for 95-LI. The in vitro dry matter digestibility (IVDMD) was higher ($p < .01$) for 95-LI (646 g/kg) relative to 33-SC (628 g/kg). Crude protein concentration was higher ($p < .01$) for 95-LI (154 g/kg) relative to 33-SC (136 g/kg). The ADF (303 g/kg DM) and aNDF (623 g/kg) were higher ($p < .01$) for 33-SC compared to 95-LI (287 and 605 g/kg respectively). The gas production potential from non-fibrous carbohydrates was 9.4% higher for 33-SC. The in vitro CH_4 production per unit of degraded dry matter (DDM) was higher ($p < .01$) for 33-SC (19.9 CH_4 ml/g), compared to 95-LI (17.9 CH_4 ml/g). The 33-SC management resulted in rest periods that were too long for the time needed for optimal regrowth. The variable SC based on the 95-LI was more favourable, because it resulted in maximum yield of dry matter with improved forage nutritive value, while decreasing CH_4 yield.

KEYWORDS

light interception, pasture management, rotational stocking, tropical grass

1 | INTRODUCTION

Ruminant production in the tropics is pasture-based as it is the most economical and practical system to feed cattle. However, pastures are often extensively exploited in degraded areas of low productivity (Dias-Filho, 2014). In addition, environmental issues related to land and water use, biodiversity conservation and emission of greenhouse gases (GHG) have been pushing the sector to become more efficient (Berchielli et al., 2012).

In Brazil, one of the main anthropogenic sources of GHG is agriculture, accounting for 32% of total emissions (Brazil, 2016). Enteric fermentations from ruminants are responsible for 66.9% of the methane (CH_4) production (Brazil, 2016). Greater animal production and reduced CH_4 emissions are enhanced by improvements in forage quality, which

can be achieved by grazing animals on less mature pastures with a lower fibre content and increased water-soluble carbohydrates concentration (Beauchemin et al., 2008; Eckard et al., 2010; Ulyatt et al., 2002). The *Urochloa brizantha* cultivar Marandu (hereafter Marandu grass) is currently the most abundant grass in Brazilian pastures. The success of this cultivar is related to its yield potential and large adaptation to the Brazilian edaphoclimatic conditions (Jank et al., 2011). However, according to Oliveira et al. (2015), its potential in not optimally utilized in production systems, owing especially to inadequate pasture management.

There is a long history of using a fixed rest period when managing tropical grasses (Euclides et al., 2014 and Da Silva et al., 2015). Traditionally, a 30-day rest period has been recommended for the management of Marandu grass (Da Cunha et al., 2012 and Ruggieri et al., 2014). However, as pasture development is influenced by edaphic and climatic conditions, the application of a fixed SC can result in grazing at an inappropriate time, reduced harvesting efficiency and lower forage nutritional quality.

Physiological guidelines, such as the light interception (LI) by the canopy has been suggested as an indicator to guide grazing events after a rest period for temperate grass already for many years, and more recently for tropical grass (Da Silva et al., 2015).

Brougham (1957) and Parsons and Penning (1988) demonstrated that temperate grasses have their maximum average growth rate when the rest period is long enough for the canopy to reach 95% of LI (95-LI), but it is relatively insensitive to regrowth duration beyond 95-LI. Studies of Brougham (1957) and Parsons and Penning (1988) also demonstrated that stems will accumulate if the duration of regrowth is sufficiently long. This will deteriorate the sward structure and will hamper grazing. According to Parsons and Penning (1988), in vitro analyses revealed that the digestibility of the herbage eaten was not affected by the regrowth duration. However, they suggested that animals were more effective in removing accumulated herbage when the regrowth period was shorter. Therefore, management guidelines should concentrate on controlling the development of stems.

In several tropical grazing studies, the maximum growth rate was reached at 95-LI and senescence and stem growth rates increased markedly after 95-LI in many grasses, including *Panicum maximum* cv. Tanzânia (Difante et al., 2009), *U. brizantha* cv. Marandu (Giacomini et al., 2009), *Panicum maximum* cv. Aruana (Zanini et al., 2012) and *Pennisetum purpureum* cv. Napier (Pereira et al., 2015). This showed consistency among a wide range of morphological types and a strong effect of light in determining plant growth (Da Silva et al., 2015).

Dos Anjos et al. (2016) concluded that shorter grazing intervals (approximately 22 days) are possible when adopting a rest period based on 95-LI compared to a fixed rest period of 30 days. They recommended 20–25 cm post-grazing heights for dairy cows, and a canopy height of 35 cm during the rest period. In addition, they observed that a rest period based on 95-LI during the rainy season promoted the highest percentage of leaves and a smaller percentage of dead material in the forage mass cut at ground level.

Although it has already been demonstrated that the maximum average growth rate and the best sward structure are both reached at 95-LI, it has not been demonstrated if this also promotes greater nutritive value of cut forage at the average height of the post-grazing residue and less CH_4 emission by animals.

We hypothesize that a 30-day rest period for rotationally stocked Marandu grass will result in greater stem elongation, leaf senescence and fibre content, whilst commencing grazing when the canopy reaches 95-LI will decrease stem elongation and leaf senescence, resulting in greater forage digestibility. Therefore, we expect greater nutritive value and reduced enteric CH_4 emissions when grazing begins at 95-LI. Thus, the aim was to evaluate the effect of two SC of rotationally stocked Marandu grass on forage chemical composition and in vitro ruminal fermentation kinetics, digestibility and CH_4 production. The two SC tested were (1) a fixed 33-day SC (33-SC) and (2) initiation of grazing based on the canopy reaching 95-LI.

2 | MATERIAL AND METHODS

All animal care and handling procedures were approved by the Embrapa Dairy Cattle Animal Care and Use Committee (Juiz de Fora, Minas Gerais, Brazil; Protocol CEUA-EGL 16/2013).

2.1 | Location and edaphoclimatic conditions

The trial was performed at Embrapa Dairy Cattle Experimental Station, Coronel Pacheco, Minas Gerais, Brazil (21°33'S, 43°16'W, altitude 410 m) from December 1, 2013 to May 3, 2014. The regional climate is classified as Cwa, i.e. mesothermic according to Köppen, with a rainy summer between October and March and a dry winter from June to September. From 2009, the pastures were stocked rotationally during the rainy season between October and March. In 2013, pastures were allowed to grow undisturbed from October to December. In December, animals grazed pastures to a residual height of 20 cm, to standardize the experimental area. Sampling started on January 20, 2014.

Weather data during the experimental period were collected by an automatic meteorological station located 200 m from the experimental site (Table 1). The soil of the experimental site was classified as Dystric Fluvisol according to the Food and Agriculture Organization (FAO, 2006), with the following chemical characteristics: Organic matter = 3.5%; P (Mehlich-1 method) = 4.2 mg/dm^3; K = 160 mg/dm^3; cation exchange capacity = 6.8 cmolc/dm^3; base saturation = 30%; and pH in water = 5.0. The pasture was fertilized with 333 kg/ha of *N* (20%), K_2O (20%) and P_2O_5 (5%) mixture at the beginning of each experimental period (January 6, February 18 and April 4, 2014) after a rainfall event.

2.2 | Experimental design and treatments

The experimental site comprised of 16 paddocks, 880 m^2 in size. The paddocks were distributed on two blocks of edaphic differences

TABLE 1 Climatic data for Coronel Pacheco (Minas Gerais, Brazil) throughout the experimental and pre-experiment periods

	Temperature (°C)					RH[a]	Precipitation	
Period	Mean	Mean maximum	Mean minimum	Maximum	Minimum	(%)	(mm)	Rainy days
Pre-experimental[b]	24.4	31.3	19.9	35.1	16.9	78.7	356.8	20
First period	24.5	32.4	18.7	34.4	16.5	72.7	61.6	9
Second period	23.7	30.7	18.8	34.7	16.4	79.2	112.4	19
Third period	22.2	28.6	18.3	32.6	8.2	81.5	108.6	17

[a]RH, relative humidity.

[b]Pre-experimental period, from December 17, 2013 to January 19, 2014; First period, from January 20 to February 22, 2014; Second period, from February 23 to March 28, 2014; Third period, from March 29 to April 30, 2014. Source: Coronel Pacheco automatic weather station (A557) of the Brazilian National Meteorological Institute (INMET).

and then arranged into four replicate blocks with each treatment allocated to each replicate block. The measurements were repeated in three experimental periods, which lasted one stoking cycle. Four paddocks were measured weekly because the replicates were in different regrowth stages and at the end of the experimental period the four replicate had been measured. Two SC were evaluated under rotational stocking: These included: (1) 33-SC, with a 30-day fixed rest period, and (2) initiation of grazing based on the canopy reaching 95-LI. The periods of occupation by animals were three days in both SC. The average rest period was 24.5-day (standard deviation of 5.2-day) for the 95-LI SC.

Pasture chemical composition and protein fractionation were evaluated in a randomized block design with repeated measures over time. For the evaluation of degradability, gas production kinetics and CH_4 production by in the in vitro gas production method, samples of each SC were organized into 12 pooled samples (blocks were joined), referring to four distinct field sample from each SC and experimental period and were evaluated in a completely randomized design with repeated measures over time.

2.3 | Pasture management and the monitoring of LI

Lactating Girolando (Holstein x Gyr) cows (548 ± 61.0 kg body weight) were used for grazing. In both treatments, the stocking rate was adjusted according to the put-and-take technique (Mott, 1960), aiming to achieve a post-grazing residual height of 20 cm.

During regrowth, a canopy analyser was used (Accupar LP-80, Decagon Devices, Pullman, WA, USA) to evaluate the LI at 20 points in each paddock every 7 days. When LI exceeded 90%, the frequency of the measurements was increased to every 3 days.

2.4 | Sampling and chemical composition

Forage samples were cut at the average height of the post-grazing residue (20 cm) at 20 points in each paddock. Samples were oven-dried at 55°C for 72 hr and processed in a Wiley mill with a 1 mm screen. Samples were analysed for dry matter (DM), ash, crude protein (CP), ether extract (EE) according to the procedures described by AOAC (1990).

The aNDF and ADF were determined based on the methods described by Van Soest et al. (1991). Lignin (sa) was determined by solubilizing the cellulose with sulphuric acid in the ADF residue (Van Soest et al., 1991). The non-fibrous carbohydrate (NFC) concentration was calculated as described by Sniffen et al. (1992).

The CP fractionation was performed according to Licitra et al. (1996). Fractions A + B1, B2, B3 and C were determined as a percentage of the total CP. These represented non-protein nitrogen and soluble proteins, insoluble proteins with intermediate degradation rates, insoluble proteins with slow degradation rates and the indigestible proteins respectively.

The in vitro dry matter digestibility (IVDMD) was determined according to Tilley and Terry (1963). Calcium and phosphorus concentrations were analysed by optical absorption spectroscopy (Silva & Queiroz, 2006) and gross energy concentration (GE) was determined by combustion in an adiabatic calorimeter (PARR 2081, Parr Instrument Company, Moline, IL, USA).

2.5 | In vitro fermentation, gas production and degradability

The fermentation of the forage samples was performed using a total of 108 flasks (100 ml) filled with filter bag (ANKOM F57), eight ml of ruminal inoculum and 72 ml of culture medium (Theodorou et al., 1994). For the evaluation of gas production over 96 hr, two laboratory replicates per each of the 24 field samples (48 flasks) were used. An additional two flasks per field sample (48 flasks) were used to assess pH of ruminal liquid, ruminal ammonia-*N* (NH_3-N), volatile fatty acid (VFA), CH_4 production and the degraded dry matter (DDM) after 24 hr of incubation. The filter bags contained 0.8 g of sample per bag. Twelve flasks containing empty filter bags were used as blank controls for both the 96-hr and the 24-hr incubation periods ($n = 6$ flasks per incubation period).

The inoculum was obtained using a composite ruminal fluid sample from four ruminally-fistulated dry cows (Holstein), fed maize silage ad libitum and 4 kg of concentrate (24% CP) per day, for 21 days prior to collection. Rumen fluid was collected from several parts of the rumen, filtered in the laboratory under continuous CO_2 injection, and kept in a water bath at 39°C. The flasks were heated in an oven to 39 °C before adding inoculum, and kept in an oven during the fermentation period.

Accumulated gas pressure in the flasks containing samples incubated until 96-hr was measured with a pressure transducer (Druck DPI 705, GE Measurement & Control, Billerica, MA, USA) at 2, 4, 6, 8, 10, 12, 14, 17, 20, 24, 28, 34, 48, 72, and 96 hr. In the other flasks, the pressure was measured at 24-hr and then 10 ml of headspace gas was sampled using a 20-ml syringe. The gas sample was immediately transferred into 6.8-ml evacuated vials (Exetainer, Labco Ltd., High Wycombe, Buckinghamshire, UK).

Pressure data were converted to volume of gas using the equation 1 (R^2 = 0.99) developed for the conditions of the Gas Production Laboratory, Embrapa Dairy Cattle Experimental Station. The bicompartmental model (equation 2) of Pell and Schofield (1993) was used to evaluate the kinetics of gas production, where: Vf_1 - maximum volume of gases from the fraction NFC, C_1 – degradation rate of fraction NFC, L – latency or lag time, Vf_2 - maximum volume of gases from the FC (fibrous carbohydrates) fraction, C_2 – degradation rate of fraction FC and T – incubation times.

$$\text{Volume of gases produced (mL)} = 0.168323 + 3.84053 * \text{Pressure (PSI)} \quad (1)$$

$$V(t) = Vf_1 / \left(1 + \exp\left(2 - 4 * C_1 * (T - L)\right)\right) + Vf_2 / \left(1 + \exp\left(2 - 4 * C_2 * (T - L)\right)\right) \quad (2)$$

The concentration of NH_3-N was determined according to the methods of AOAC (1990). Liquid sample to determine VFA concentrations were first centrifuged at 5,000 rpm for 10 min and then analysed using a high-performance liquid chromatography (Waters Alliance Chromatograph e2695, Waters Technologies of Brazil LTDA, Barueri, SP, Brazil). CH_4 concentration was determined in a gas chromatograph (model 7820A Agilent Technologies, Santa Clara, CA, USA) equipped with injectors coupled to a 0.5 cm^3 loop and automated valve, flame ionization detector (FID) with Plot HP-Al/M and HP-MolSiv Megabore capillary columns. After a 24-hr fermentation period, the F57 filter bags were washed in water until the residual water was clean and the DDM was determined by drying at 105°C until a constant weight was reached.

2.6 | Calculations

Calculations of VFA and CH_4 yield were performed using Equations 3 and 4 respectively. To calculate the production of CH_4 ml/g DDM, the last item of Equation 4 (g DM) was replaced by DDM (g). To convert the values to energy, the factor of 39.54 kJ/L of CH_4 was applied (Santoso et al., 2007).

$$VFA\ mmol/g\ DM = ((mL\ of\ liquid \times VFA\ mmol/mL) - (mL\ of\ blank \times VFA\ of\ blank\ mmol/mL))\ /\ g\ DM \quad (3)$$

$$CH_4\ mL/g\ DM = ((mL\ of\ gases\ after\ 24\ hr \times CH_4\ mL/mL) - (mL\ of\ gases\ after\ 24\ h\ of\ blank \times CH_4\ of\ blank\ mL/mL))\ /\ g\ DM \quad (4)$$

The partitioning factor (PF) and the rumen microbial protein production (RMPP) were calculated according to Blümmel et al. (1997) using Equations 5 and 6 respectively.

$$PF = \text{mg of DDM / mL of gas after 24 h} \quad (5)$$

$$RMPP\ \text{mg/g DM} = \text{mg DDM} - (\text{mL gas} \times 2.2\ \text{mg/mL}) \quad (6)$$

2.7 | Statistical analysis

The in vitro gas production kinetics parameters were estimated by the model of Schofield et al. (1994) using the Gauss-Newton algorithm to adjust for non-linear regression using PROC NLIN (SAS, 2016).

Data of chemical composition, protein fractionation and data generated in the in vitro gas production trial were analysed using PROC MIXED of SAS (2016). Where appropriate, Fisher's test was used and the Pearson's correlation coefficient was calculated. The statistical significance was considered when $p \leq .05$.

3 | RESULTS

3.1 | Chemical composition, in vitro digestibility and CP fractionation

There was no interaction between the SC and the experimental period for the forage chemical composition variables ($p > .05$). The levels of DM, OM, EE, lignin (sa), NFC, Ca and P in forage were not influenced ($p > .05$) by the SC. The levels of aNDF and ADF were 3.0% and 5.2% higher ($p < .01$), respectively, for 33-SC compared to 95-LI. The IVDMD and CP were 2.8% and 11.3% lower ($p < .01$), respectively, for 33-SC compared to 95-LI (Table 2).

The DM concentration was 19.3% higher ($p < .01$) in the 1st period compared to the 3rd period. The NFC concentration was 22.1% higher ($p < .01$) in the 1st period compared to the 3rd period when 33-SC was used and it was 14.9% higher ($p < .01$) in the 1st period compared to the average of the 2nd and 3rd periods when 95-LI was used. The IVDMD increased ($p = .04$) throughout the experimental periods by 1.8% from the 1st to the 2nd period and 1.2% from the 2nd to the 3rd period. The CP concentration increased ($p < .01$) throughout the experimental periods and in the 1st period the CP was 20.5% lower than the average of the 2nd and 3rd periods (Table 2).

The CP fractionation was not influenced ($p > .05$) by SC and average values of 342.1, 363.4, 250.1 and 40.4 g/kg CP were observed for the fractions A + B1, B2, B3 and C respectively (Table 2).

TABLE 2 Dry matter (DM), organic matter (OM), ether extract (EE), neutral (aNDF) and acid (ADF) detergent fibre, lignin, non-fibrous carbohydrates (NFC), calcium (Ca), phosphorus (P), in vitro dry matter digestibility (IVDMD), crude protein (CP) and crude protein fractionation of *Urochloa brizantha* cv. Marandu under a fixed regrowth period of 30 days (33-SC) or when the canopy reaches 95% light interception (95-LI)

	33-SC			95-LI				*p* - value		
Item	1st Period	2nd Period	3rd Period	1st Period	2nd Period	3rd Period	SEM[a]	SC[b]	Period	SC x Period
DM (g/kg)	253.1a	227.2ab	204.7b	251.3a	231.7ab	202.2b	13.2	>.50	<.01	>.50
OM (g/kg DM)	903.6	907.5	911.6	908.3	911.3	911.9	3.1	.26	.19	>.50
EE (g/kg DM)	21.9	22.2	22.5	19.4b	25.7a	21.1ab	2.0	>.50	.27	.29
aNDF (g/kg DM)	627.0	618.6	624.4	609.8	607.6	596.5	8.6	<.01	>.50	>.50
ADF (g/kg DM)	303.0	292.8	313.0	290.1	284.8	286.4	6.3	<.01	.13	.21
Lignin (g/kg DM)	34.4	35.1	38.1	36.0	37.4	35.6	1.8	>.50	>.50	.36
NFC (g/kg DM)	141.0a	126.6ab	109.8b	145.5a	124.1b	123.5b	8.1	>.50	<.01	>.50
Ca (g/kg DM)	10.5	10.6	10.9	10.6	10.6	10.6	0.1	>.50	>.50	>.50
P (g/kg DM)	1.1	1.2	1.4	0.9	1.6	1.4	0.2	>.50	.14	>.50
IVDMD (g/kg DM)	616.4b	632.4a	635.3a	637.0b	644.2ab	656.8a	8.2	<.01	.04	>.50
CP (g/kg DM)	113.8b	140.1a	155.0a	133.6b	154.5a	172.9a	9.4	<.01	<.01	>.50
A + B1 (g/kg CP)	362.6	306.9	352.3	315.7	311.9	403.2	40.7	>.50	.26	>.50
B2 (g/kg CP)	335.9	385.3	388.1	376.6	366.0	328.6	37.6	>.50	>.50	>.50
B3 (g/kg CP)	257.5	256.3	223.8	255.8	274.6	232.7	16.6	>.50	.06	>.50
C (g/kg CP)	44.1	38.8	35.8	40.7	47.6	35.5	4.4	>.50	.20	>.50

Note: Lowercase letters compare grazing cycles for grazing intervals strategy. Means followed by distinct letters differ by Fisher's test ($p < .05$).

[a]Standard error of the mean.

[b]Stocking cycle.

TABLE 3 Maximum volumes of gases from non-fibrous carbohydrate (Vf_1-NFC) and fibrous carbohydrate fractions (Vf_2-FC), fermentation rates of non-fibrous carbohydrates (C_1-NFC) and fibrous carbohydrates (C_2-FC), and latency (L) of in vitro fermentation of *Urochloa brizantha* cv. Marandu under a fixed regrowth period of 30 days (33-SC) or when the canopy reaches 95% light interception (95-LI)

	33-SC			95-LI		
Item	1st Period	2nd Period	3rd Period	1st Period	2nd Period	3rd Period
Vf_1-NFC (ml)	89.8	91.8	89.7	73.2	82.3	90.2
C_1-NFC (ml/h/total ml)	0.06	0.05	0.06	0.07	0.06	0.06
L (h)	5.2	4.8	4.9	5.2	4.7	5.2
Vf_2-FC (ml)	124.8	125.0	129.1	129.5	122.7	133.1
C_2-FC (ml/h/total ml)	0.02	0.01	0.01	0.02	0.01	0.01
R^2	0.99	0.98	0.98	0.98	0.99	0.97

Note: R^2 - coefficient of determination.

3.2 | In vitro fermentation kinetics

An interaction ($p = .05$) was observed between SC and the experimental period for gases produced during in vitro fermentation. For this reason, the fit of the equation of Schofield et al. (1994) was sliced for each experimental period, with R^2 values varying from 0.97 to 0.99 (Table 3).

The Vf_1-NFC increased throughout the experimental period for 95-LI; however, it was not influenced by the experimental period for 33-SC. The Vf_1-NFC was 18.4 and 10.4% higher for 33-SC when compared to 95-LI in the 1st and 2nd period respectively. Vf_2-FC was similar between the SC and 6.6% lower in the second period compared to the other periods when 95-LI was used (Table 3).

The C_1-NFC was 11.1% higher in the 1st period in the average of the two SC. The C_2-FC was 6.7 and 17.6% higher in the 1st period for 33-SC and 95-LI, respectively, and 11.8% higher for 95-LI when compared to 33-SC in the 1st period (Table 3).

The L was similar between SC in all periods, with a maximum difference of 5.2% in the 3rd period. Within 33-SC, the L was 6.8% higher in the 1st period compared to the other periods. Within 95-LI,

L was 9.7% lower in the second period compared to the other experimental periods (Table 3).

3.3 | VFA, pH and NH_3-N

No interaction between SC and experimental period was observed for total VFA, acetate, propionate and butyrate production, acetate/propionate (A/P) ratio, pH and NH_3-N concentration ($p > .05$). Fermentation parameters after 24 hr were not influenced by SC ($p > .05$) (Table 4).

The pH of the ruminal liquid was 1.1% lower ($p < .01$) in the 1st period compared to the average of the 2nd and 3rd period when 33-SC was used. When 95-LI was used, the pH of the ruminal liquid was 0.87% lower ($p < .01$) in the 1st period compared to the 3rd period, but these were not different compared to the 2nd period. The production of butyrate was 18.2% lower in the 1st and 2nd period for 95-LI and 33-SC, respectively, compared to the respective averages of the 3rd period. The NH_3-N was 28.3 and 17.3% higher in the 3rd period for 33-SC and 95-LI, respectively, compared to the averages of the 1st and 2nd periods (Table 4).

The DDM after 24 hr was 5.8% higher ($p = .03$) for 95-LI when compared to 33-SC, but the gas production was 4.13% lower ($p = .03$). Neither DDM nor gas production was influenced ($p > .05$) by the experimental periods (Table 5).

The CH_4 production in ml/g DM was not influenced by SC, but it was 16.4% lower ($p < .01$) in the 1st period compared to the 3rd period when 95-LI was used. The CH_4 yield (ml/g DDM) was 9.8% higher ($p < .01$) for 33-SC compared to 95-LI and 14.8% lower ($p = .01$) in the 1st period when 95-LI was used (Table 5).

The GE and the loss of energy in MJ/kg DM as CH_4 were not influenced ($p > .05$) by SC. The loss of energy as CH_4 was 12.9% lower ($p < .01$) in the 1st period compared to the 3rd period when 95-LI was used. The RMPP and PF were 18.8 and 9.8% higher ($p < .01$) when 95-LI was used, but were not influenced by the experimental periods ($p > .05$) (Table 5).

4 | DISCUSSION

4.1 | Forage composition

The hypotheses were accepted, the use of 95-LI as criterium to start grazing Marandu grass decreased the accumulation of fibre, improved nutritional quality and reduced CH_4 yield compared with fixed 33-day SC.

Changes in the pattern of forage accumulation due to stem elongation and leaf senescence may occur if SC allows a LI greater than 95% (Da Silva et al., 2015; Parsons & Penning, 1988; Pedreira et al., 2009; Sousa et al., 2013). These changes reduce the proportion of leaves and increase the proportion of stem and dead material in the forage (Nave et al., 2010). This explains the higher CP concentration and lower aNDF and ADF concentrations when the SC was managed based on 95-LI, since the regrowth period for 33-SC was 19.2% longer and the LI was 2.4% greater than for 95-LI. The hypothesis that a 30-day rest period for Marandu grass may exceed the time for the canopy to reach 95-LI was accepted. Moreira et al. (2004) studied Giant star grass (*Cynodon plectostachyus* Pilger) under similar conditions and observed 53.4 and 69.5% more CP, 13.5 and 17.3% less aNDF, and 26.0 and 28.1% less ADF in leaves compared to stems

TABLE 4 The pH, total volatile fatty acids (VFA), acetate, propionate, butyrate, acetate/propionate ratio and ammoniacal nitrogen (NH_3-N) from the liquid of in vitro fermentation of *Urochloa brizantha* cv. Marandu under a fixed regrowth period of 30 days (33-SC) or when the canopy reaches 95% light interception (95-LI)

	33-SC			95-LI				*p* - value		
Item	1st Period	2nd Period	3rd Period	1st Period	2nd Period	3rd Period	SEM[a]	SC[b]	Period	SC x Period
pH	6.75b	6.81a	6.84a	6.76b	6.80ab	6.82a	0.02	>.50	<.01	>.50
VFA (mmol/g DM)	1.37	1.37	1.49	1.25	1.49	1.51	0.11	>.50	.28	>.50
Acetate (mmol/g DM)	0.76	0.77	0.83	0.70	0.85	0.84	0.07	>.50	.36	>.50
Propionate (mmol/g DM)	0.41	0.42	0.44	0.37	0.44	0.45	0.04	>.50	>.50	>.50
Butyrate (mmol/g DM)	0.19ab	0.18b	0.22a	0.18b	0.20ab	0.22a	0.01	>.50	<.01	.21
Acetate/ Propionate	1.88	1.86	1.88	1.87	1.91	1.87	0.04	>.50	>.50	>.50
NH_3-*N* (mg/100ml)	27.74b	32.64b	42.09a	31.85b	31.50b	38.32a	17.01	>.50	<.01	.23

Note: Lowercase letters compare grazing cycles for grazing intervals strategy. Means followed by distinct letters differ by Fisher's test ($p < .05$).

[a]Standard error of the mean.

[b]Stocking cycle.

TABLE 5 Dry matter disappearance (DDM) and gas production after 24 hr, methane production (CH_4) per gram of incubated dry matter (DM) and degraded dry matter (DDM), gross energy (GE), energy loss as methane (E-CH_4), rumen microbial protein production (RMPP) and partitioning factor (PF) of *Urochloa brizantha* cv. Marandu under a fixed regrowth period of 30 days (33-SC) or when the canopy reaches 95% light interception (95-LI)

	33-SC			95-LI				*p* - value		
Item	1st Period	2nd Period	3rd Period	1st Period	2nd Period	3rd Period	SEM[a]	SC[b]	Period	SC x Period
DDM (mg/g)	402.9	397.5	405.2	429.3	407.2	442.9	13.1	.03	.24	>.50
Gas production (ml/gDM)	115.1	113.0	113.6	107.5	107.3	112.8	4.5	.03	>.50	.38
CH_4 (ml/gDM)	7.5	8.1	8.3	6.9b	7.7ab	8.2a	0.4	.15	<.01	>.50
CH_4 (ml/gDDM)	18.6	20.4	20.6	16.0b	19.0a	18.6a	1.1	<.01	.01	>.50
GE (MJ/kgDM)	17.1	17.4	17.4	17.3	17.6	17.5	0.1	.06	.07	>.50
E-CH_4 (MJ/kgDM)	0.30	0.32	0.33	0.27b	0.30ab	0.32a	0.02	.15	<.01	>.50
RMPP (mg/gDM)	149.6	149.0	155.3	192.7	171.1	194.9	15.6	<.01	>.50	>.50
PF (mgDDM/ml gas)	3.5	3.5	3.6	4.0	3.8	4.0	0.2	<.01	>.50	>.50

Note: Lowercase letters compare grazing cycles for grazing intervals strategy. Means followed by distinct letters differ by Fisher's test ($p < .05$).

[a]Standard error of the mean.

[b]Stocking cycle.

and the dead material respectively. Similarly, Porto et al. (2009) reported that the leaves of Marandu grass managed under a rotational stocking system had 100% more CP and 13.3% less aNDF than the stems. These results have proved the hypothesis that management which increases the leaf proportion of forage reduces its fibre fraction and increases CP concentration.

The reduction of aNDF (2.9%) and ADF (3.2%) concentrations by using the 95-LI criterion to interrupt the regrowth process compared with a fixed regrowth period of 26 days was also found by Voltolini et al. (2010) for Elephant grass (*Pennisetum purpureum* Schum.). Nave et al. (2010) observed a reduction of 3.0% aNDF and 4.3% ADF in the stem of Xaraés grass (*Urochloa brizantha* cv. Xaraés) when the criterion of 95-LI was used and compared to a 28-day of regrowth period, although no reduction in the concentration of aNDF and ADF for the whole plant was reported.

Voltolini et al. (2010) did not observe differences in CP concentration between Elephant grass harvested with 95-LI or a 26-day rest period. On the other hand, Nave et al. (2010) also observed that the CP concentration of Xaraés grass was 15.9% higher when harvested with 95-LI compared to a 28-day rest period (138 vs. 116 g/kg DM), but did not observe differences in the CP concentration of the leaves. Therefore, it can be inferred that higher CP values observed for 95-LI SC are associated with a higher proportion of leaves in the forage mass.

The higher IVDMD values for 95-LI may also be associated with the higher proportion of leaves in the forage mass. The positive correlation ($r = 0.77$; $p < .01$) between the leaf proportion and the IVDMD observed by Moreira et al. (2004) in star grass during the summer support this hypothesis. In addition, Nave et al. (2010) observed that IVDMD was 4.8% higher in leaves than in stems, and 2.0% higher in leaves and stems when the forage was harvested at 95-LI compared to 28 days fixed rest for Xaraés grass. These responses indicate that the IVDMD of the plant can be influenced by the IVDMD of individual plant components and their proportions. In addition, they also proved the hypothesis that applying 95-LI as criterium to start grazing resulted in lower fibre and higher intracellular content concentrations and increased digestibility of forage.

For Marandu grass cut every 28 days, Pequeno et al. (2015) found values of 139, 555, and 654 g/kg DM for CP, aNDF and in vitro organic matter digestibility, respectively, similar to that of the present study when the forage was harvested with a 30-d rest period. Using the same grass, Sá et al. (2010) reported a linear reduction of the B1 + B2 fraction associated with a linear increase of the B3 and C fractions as a function of the advancement of plant maturity. However, the rest periods were 28, 35 and 54 days, with a larger difference compared with the present study. Perhaps the difference of 6 days on average, in the present study, was not enough to influence the forage protein fractions. For Elephant grass managed with 95-LI, Danés et al. (2013) reported similar values (213, 92, 359, 288 and 48 g/kg CP) for fractions A, B1, B2, B3 and C respectively.

Variation in CP, IVDMD and NFC throughout the experimental periods may be associated with the pre-experimental conditions of the area. Prior to the start of the experiment, the area was not fertilized or grazed for a year and stems and dead leaves accumulated. Several authors (Huber et al., 1999; Paiva et al., 2012; Santos et al., 2011) have also reported changes in the forage structure with grazing. Therefore, it is expected that grazing increases the proportion of leaves in the forage and changes the chemical composition. The most sensitive nutritional component is CP, which according to Porto et al. (2009) is the variable of greatest

discrepancy between leaf and stem for Marandu grass. In addition, Johnson et al. (2001) reported a linear increase in CP concentration and in in vitro OM digestibility with increasing nitrogen fertilization from 0 to 157 kg N/ha in tropical grasses. Therefore, nitrogen fertilization and the residual effect of fertilization may explain the increase in CP and IVDMD throughout the experimental periods.

The NFC reduction throughout the experimental periods was associated with an increase in CP, since no changes were observed in the fibrous fractions throughout the periods. This reduction, therefore, does not represent losses in nutritive value that supports the increase in IVDMD.

The 95-LI criterion was more adequate to obtain forage with a higher proportion of leaves, that are more digestible, compared to 33-SC with less leaves (Nave et al., 2010). This was associated with a shorter regrowth time for 95-LI in the edaphoclimatic conditions of the present study. However, the simple reduction of the rest period and the maintenance of this with fixed intervals can improve the nutritive value of the forage, but does not guarantee the maximum average growth rate of the pasture, which only occurs when the pasture reaches 95-LI as demonstrated by Parsons and Penning (1988).

In addition, the large variation in regrowth showed that is not possible to determine an ideal fixed rest period. The mean regrowth time to 95-LI was 24.5 days, the maximum regrowth time was 36 days and the minimum regrowth time was 15 days. There was a positive correlation between LI and rest period, but with a low correlation coefficient ($r = 0.44$; $p < .01$). However, a strong positive correlation ($r = 0.74$; $p < .01$) between LI and pre-grazing height was observed, suggesting that pre-grazing height can be used to determine the end of the rest period in practice. The mean pre-grazing height for 95-LI was 32.6 cm. The logical recommendation from these results is that grazing of Marandu grass should start when the canopy is between 30 and 35 cm in height.

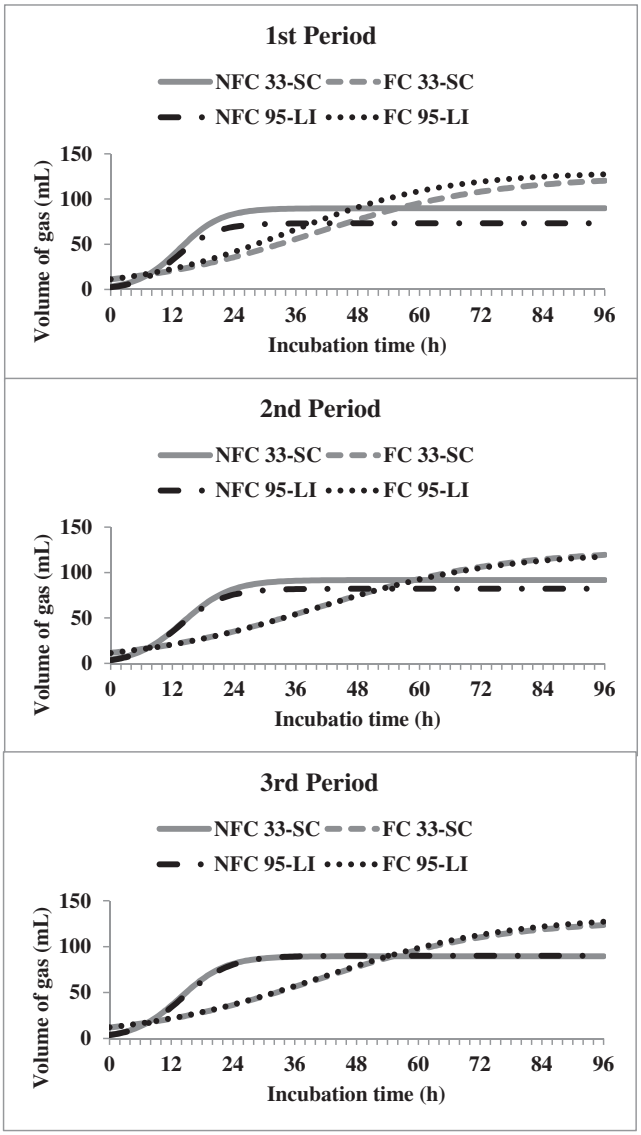

FIGURE 1 Gas production from non-fibrous carbohydrates (NFC) and fibrous carbohydrates (FC) of *Urochloa brizantha* cv. Marandu under two criteria to start grazing in three consecutive periods (33-SC - fixed regrowth period of 30 days; 95-LI - grazing allowed when the photosynthetically active radiation intercepted by the canopy reached 95%)

4.2 | In vitro fermentation kinetics

The gas production curves from NFC and FC were similar for both SC in all periods (Figure 1). The L in the present study ranged from 4.66 to 5.21 hr, which were less than the 12.9 hr observed by Sá et al. (2011) for Marandu grass harvested after 28 days of regrowth, However, the values in this study were higher than the value of 2.68 hr observed by Velásquez et al. (2009) for Marandu grass harvested from January to March, with an average regrowth of 35 days. Differences in L between studies may be explained by the aNDF concentration in the forage. In the study by Sá et al. (2011), the forage aNDF was 646 g/kg, higher than the average aNDF observed in this study (623 g/ kg for 30-SC and 604 g/ kg for 95-IL). In the study by Velásquez et al. (2009) the forage was cut at 25 cm height, higher than the present study (20 cm), which supports lower forage aNDF (512 g/ kg) and low L compared with the present study.

The volume of gas produced from NFC increased rapidly due to the high values of C_1-NFC, as expected for the NFC fraction, and the asymptote of the curve was established after approximately 24 hr, indicating that this fraction was completely degraded in this period.

The NFC fraction of forages usually consists of organic acids, monosaccharides (glucose and fructose), disaccharides (sucrose and maltose) and polysaccharides (starch and fructan) (Smith, 1972). These sugars are intermediate metabolites of metabolic pathways and reserve carbohydrates of the plant (Stryer, 1975). Tropical grasses mainly accumulate starch as a reserve carbohydrate (Smith & Grotelueschem, 1966). However, the proportion of soluble carbohydrates in plant tissues and organs is dependent on many factors, such as those related to environmental conditions, nutritional status and to the physiological stage of the plant (George et al., 1989; Reis et al., 1985).

The variation in the proportion of carbohydrates contained in the NFC fraction may explain the higher C_1-NFC observed in the 1st period, since organic acids and simple sugars have higher fermentation rates than polysaccharides such as starch (Sniffen et al., 1992). This would also explain, in part, the increase in Vf_1-NFC throughout the three experimental periods for 95-LI, because the volume of gases produced from organic acids is ⅓ to ⅔ of the volume produced from starch (Pell et al., 2000). Simple sugars also produce less gas than starch because of the lower amount of glucose per gram of substrate (Hall & Herejk, 2001). According to Satter and Slyter (1974), the microbial yield of energy substrates linearly increased with the elevation of CP levels up to the limits of about 130 to 140 g/kg. After these limits, the excess amino acids are subject to deamination and the carbon skeletons can then be fermented to produce VFA and gases. Therefore, the effect of NFC reduction on Vf_1-NFC in the 2nd and 3rd period may have been compensated by the fermentation of carbon skeletons from deamination of protein that exceeded the microbial demand for growth. The higher value of NH_3-N after 24 hr of fermentation during the 3rd period was coherent with this finding.

The Vf_1-NFC was not different between the experimental periods within the 33-SC treatment, but was higher compared to 95-LI in the 1st and 2nd period. According to Blümmel and Ørskov (1993) and Blümmel et al. (1999), in vitro gas production reflects the fermentation of the substrate to VFA, but part of the fermented substrate can be incorporated as microbial cells (Blümmel et al., 1997). The in vitro partitioning of the substrate between VFA and microbial cells is not constant and they compete for the substrate (Blümmel et al., 2003). The higher value of Vf_1-NFC for 33-SC in the 1st and 2nd periods can be explained by the higher partitioning of substrate for VFA production. This higher partitioning for VFA production may have occurred due to the lower CP concentration observed for 33-SC, reducing the availability of nitrogen after the first 24 hr of fermentation, which supports the lower values of RMPP and PF for 33-SC. Vf_1-NFC did not differ in the 3rd period between 33-SC and 95-LI, which likely occurred because CP concentration exceeded the microbial assimilation capacity, increasing protein and VFA fermentation, as discussed above.

The Vf_2-FC was higher than Vf_1-NFC for both SC treatments, which reflects the high proportion of aNDF found in tropical forages. C_2-FC was lower than C_1-NFC as expected, and the asymptote of the gas production curve from FC was established between 84 and 96 hr. C_2-FC was higher in the 1st period, most likely due to a faster microbial growth caused by the rapid degradation rate of NFC fraction in this period, demonstrated by the high C_1-NFC. Hall and Herejk (2001) and Broderick and Radloff (2004) demonstrated the effect of the rapid fermentation rate of carbohydrates, such as sugar, on microbial growth rate and fibre degradation.

4.3 | VFA and NH_3-N

The proportion of VFA (acetate = 56%, propionate = 30% and butyrate = 14%) and the A/P ratio (1.88) of Marandu grass fermented for 24 hr was similar to that of diets with high NFC (20% forage and 80% concentrate) as proposed by Annison and Armstong (1969). This probably occurred because after 24 hr fermentation, 68.8% of the gas produced originated from NFC and only 31.2% from FC. In contrast, if the evaluation of VFA was performed after 96 hr of fermentation, the VFA profile would change, since 53.2% of the gas produced after 96 hr originated from the fermentation of FC and 41.4% of NFC. Gemeda and Hassen (2014) observed A/P ratio values above those observed in this study, varying from 2.20 to 3.55 for 16 different species of tropical grasses. However, the evaluation of VFA concentration was performed after 72 hr of fermentation. Danés et al. (2013) reported higher values for A/P ratio (3.33) for cows grazing *Pennisetum purpureum* managed with 95-LI and supplemented with 5 kg concentrate (on a DM basis). This suggests that the evaluation of VFA concentration after 24 hr in vitro does not reflect well what occurs in vivo, probably due to the lack of removal of VFA by absorption or passage.

The higher DDM after 24 hr of fermentation for 95-LI was possibly associated with the lower concentration of aNDF and ADF when this SC treatment was used, since most of the indigestible material after 24 hr was FC. Conversely, the gas production after 24 hr was lower for 95-LI, increasing the PF for this SC. This can be explained by a higher proportion of substrate assimilated by microbial cells and a lower proportion of substrate used for VFA and gas production when 95-LI was used.

The reduction of total VFA was not observed in the present study, suggesting a stoichiometric distortion between the total VFA production and gas production.

Cone (1998) proposed that the increase of CP in substrate could increase NH_3 production and NH_4 formation. Formation of NH_4 from NH_3 would decrease the available H^+ and prevent the buffering mechanism of VFA by bicarbonate from releasing CO_2 in the gas phase which would result in less gas production.

The NH_3-N concentration was not different between the SC treatments, despite the higher CP concentration for 95-LI. In addition, the hypothesis of decreased the gas production by NH_4 formation, reported by Cone (1998), was not confirmed by Blümmel et al. (1999) for substrates with less than 400 g/kg CP. Further research is needed to understand why the PF increased for 95-LI without a reduction in VFA production. This phenomenon may have occurred because there is some unknown metabolic process in the fermentation of amino acids; the metabolic pathway has only been described for valine, leucine and isoleucine, which are converted into branched-chain fatty acids.

The RMPP estimate was higher for 95-LI due to the higher PF. This seems to be in agreement with the NH_3-N concentrations, because in spite of the higher CP concentration for 95-LI, the concentration of NH_3-N did not differ between the SC, suggesting a greater assimilation of nitrogen by the microorganisms. The lower RMPP observed for 33-SC did not occur due to nitrogen deficiency, since NH_3-N concentrations were above the optimal value 5 mg/dl required for microbial protein synthesis (Satter & Slyter, 1974). This suggests that the forage managed with 95-LI provided more energy for microbial synthesis.

Regardless of SC, the high NH_3-N concentrations reflect an excess of nitrogen not used for microbial synthesis (Kolver et al., 1998; Russell et al., 1983), which probably occurred due to the high CP concentration and the low concentrations of NFC that are common in intensively managed tropical pastures (Danés et al., 2013).

The advancement of plant maturity generally results in decreased CH_4 production when expressed in ml/g DM due to the reduced total gas production from lower substrate degradation (Navarro-Villa et al., 2011; Purcell et al., 2011; Teixeira et al., 2015). However, there is some divergence between studies when CH_4 production was expressed in g/kg of DDM. Some studies showed no effect of plant maturity (Navarro-Villa et al., 2011; Ribeiro Junior et al., 2014; Teixeira et al., 2015), while others reported an increase in CH_4 production with advanced plant maturity (Purcell et al., 2011). In the present study, the CH_4 yield (ml/g DDM) was lower for 95-LI, and this can be explained by the higher DDM value after 24 hr for 95-LI. Therefore, if the DDM after 24 hr was higher and the CH_4 production per kg DDM was lower in the present study, it is possible that management with 95-LI has the potential to reduce the CH_4 intensity (per unit of animal product) due to improved forage quality for ruminant feeding, as suggested by McGeough et al. (2010).

Singh et al. (2012) found CH_4 values higher than in the present study, being 16.2 g/kg DM and 25.2 g/kg DDM for *Pennisetum purpureum* (elephant grass) and 11.5 g/kg DM and 28.3 g/kg DDM for *Panicum maximum* (Guinea grass), both in the pre-flowering stage. Teixeira et al. (2015) used a respirometric chamber and found higher values for CH_4 production compared to the present study, ranging from 13.3 to 18.9 g/kg DM and 25.5 to 29.1 g/kg DDM for sheep fed *P. purpureum* harvested from 56 to 112 days of growth. For *Andropogon gayanus* (Gamba grass) harvested between 56 and 140 days of growth and conserved as hay, values of CH_4 ranged from 57.7 to 60.4 g/kg DDM after 72 hr of incubation in an in vitro trial (Ribeiro Junior et al., 2014). The lower CH_4 production found in the present study compared to those described above suggests that shorter rest periods may reduce CH_4 emission by grazing animals.

Adoption of this management practice can improve animal production in palisade grass rotational grazing systems.

5 | CONCLUSION

The usual management of Marandu grass with fixed rest periods of 30 days was found to exceed the necessary rest period for optimum grazing conditions to promote greater ruminant nutrition and mitigate CH_4 emissions. The 33-SC management resulted in reduced forage nutritive value and increased CH_4 yield compared to 95-LI management. With 95-LI, forage had a reduced fibre content, increased CP concentration and increased in vitro DDM. The practical recommendations as a result of this study is to manage Marandu grass based on a 95-LI SC, beginning grazing when the canopy is between 30 and 35 cm high.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

André Morais Moura: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (supporting); Investigation (lead); Methodology (lead); Project administration (supporting); Resources (supporting); Software (supporting); Supervision (supporting); Validation (supporting); Visualization (supporting); Writing-original draft (lead); Writing-review & editing (supporting). **Thierry Ribeiro Tomich:** Conceptualization (supporting); Data curation (supporting); Formal analysis (lead); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (lead); Resources (lead); Software (lead); Supervision (lead); Validation (lead); Visualization (lead); Writing-original draft (supporting); Writing-review & editing (lead). **Luiz Gustavo Ribeiro Pereira:** Conceptualization (supporting); Data curation (supporting); Formal analysis (supporting); Funding acquisition (equal); Investigation (supporting); Methodology (equal); Project administration (equal); Resources (lead); Software (lead); Supervision (equal); Validation (equal); Visualization (equal); Writing-original draft (supporting); Writing-review & editing (equal). **Domingos Sávio Campos Paciullo:** Conceptualization (equal); Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Supervision (supporting); Validation (supporting); Writing-original draft (supporting); Writing-review & editing (equal). **Carlos Augusto Gomide:** Conceptualization (supporting); Funding acquisition (supporting); Methodology (supporting); Resources (supporting); Writing-review & editing (supporting). **Lucio C Gonçalves:** Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Methodology (equal); Supervision (equal); Validation (equal); Visualization (equal); Writing-review & editing (equal).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

André Morais Moura https://orcid.org/0000-0002-3202-8767
Thierry Ribeiro Tomich https://orcid.org/0000-0002-8788-5715

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