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# **Beef cattle responses to pre-grazing sward height and low level of energy supplementation on tropical pastures**

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# Abstract

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The objective of this study was to investigate the effects of energy supplementation and pre-grazing sward height on grazing behavior, nutrient intake, digestion, and metabolism of cattle in tropical pastures managed as a rotational grazing system. Eight rumen-cannulated Nellore steers (24 mo of age; 300 ± 6.0 kg body weight [BW]) were used in a replicated 4 × 4 Latin square design. Treatments consisted of two levels of energy supplementation (0% [none] or 0.3% of BW of ground corn on an as-fed basis) and two pre-grazing sward heights (25 cm [defined by 95% light interception (**LI**)] or 35 cm [defined by ≥ 97.5% LI]) constituting four treatments. Steers grazed Marandu Palisadegrass [*Brachiaria brizantha* Stapf. cv. Marandu] and post-grazing sward height was 15 cm for all treatments. Forage dry matter intake (DMI) was increased (*P* = 0.01) when sward height was 25 cm (1.86% vs. 1.32% BW) and decreased (*P* = 0.04) when 0.3% BW supplement was fed (1.79% vs. 1.38% BW). Total and digestible DMI were not affected by energy supplementation (*P* = 0.57) but were increased when sward height was 25 cm (*P* = 0.01). Steers grazing the 25-cm sward height treatment spent less time grazing and more time resting, took fewer steps between feeding stations, and had a greater bite rate compared with 35-cm height treatment (*P* < 0.05). Energy supplementation reduced grazing time  $(P = 0.02)$  but did not affect any other grazing behavior parameter  $(P = 0.11)$ . Energy supplementation increased (*P* < 0.01) diet dry matter digestibility but had no effect on crude protein and neutral detergent fiber digestibilities (*P* = 0.13). Compared with 35-cm pre-grazing sward height, steers at 25 cm presented lower rumen pH (6.39 vs. 6.52) and greater rumen ammonia nitrogen (11.22 vs. 9.77 mg/dL) and N retention (49.7% vs. 20.8%, *P* < 0.05). The pre-grazing sward height of 25 cm improved harvesting efficiency and energy intake by cattle, while feeding 0.3% of BW energy supplement did not increase the energy intake of cattle on tropical pasture under rotational grazing.

**Key words:** corn, grazing management, supplementation, tropical pastures

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#### **Abbreviations**



# Introduction

Forage intake of grazing cattle is regulated primarily by the harvesting process (Chacon and Stobbs, 1976; Hodgson et al., 1977) and is one of the most important determinants of animal performance (da Silva et al., 2013). When feed reaches the rumen, intake will start being regulated by forage physical effects or rumen fill (Conrad et al., 1964). Sward structure of tropical grasses affects forage harvesting efficiency by grazing cattle (Burns and Sollemberger, 2002; da Silva et al., 2013) and it might have an impact on forage intake as great as rumen fill (Hodgson et al., 1977).

The concept of an ideal time to begin grazing using 95% light interception (**LI**) as an appropriate criterion has been successfully applied to tropical grasses (Giacomini et al., 2009a; Geremia et al., [201](#page-9-0)8). In several studies conducted with different tropical grasses, a range of sward height values that correlated to 95% LI resulted in greater forage harvest efficiency (Giacomini et al., 2009a, 2009b; da Silva et al., 2013). Sward height values correlated with LI greater than 95% often create a severe horizontal barrier (Giacomini et al., 2009b; da Silva et al., 2013) (i.e., greater amount of stems and reduced leaf:stem ratio), which may reduce grazing efficiency by decreasing bite area, bite rate, and consequently forage intake (Benvenutti et al., 2009).

Performance of cattle grazing tropical grasses is far less than the animal's genetic potential for growth, even on well-managed pastures and this can be attributed primarily to limited energy intake (de Souza et al., 2017). Feeding energy concentrates provides a means to increase energy intake, stocking rate, and cattle performance (Vendramini et al., 2006; Cappellozza et al., 2014), but the interaction between energy supplementation and grazing management has not been studied in tropical grasses. We hypothesized that for Marandu Palisadegrass pre-grazing sward height of 35 cm can limit the forage intake and nutrient digestibility compared with 25-cm pre-grazing sward height. We also hypothesized that energy supplementation would increase energy intake and N retention of grazing cattle and could reduce the negative effect of higher pre-grazing sward height over the intake and nutrients digestibility. The objective of this study was to determine the influence of energy supplementation and pregrazing sward height on cattle grazing behavior, nutrient intake, digestion, and metabolism.

# Materials and Methods

This study was conducted in Piracicaba, São Paulo, Brazil (22°42′S, 47°37′W, 546 m a.s.l.). All procedures involving animals were approved and followed all the guidelines required by the Animal Care and Use Committee of the "Luiz de Queiroz" College of Agriculture, University of São Paulo.

#### **Animals and experimental area**

The experiment was conducted during the months of February to April 2011. Weather data for the experimental period were recorded at a weather station located 1 km from the experimental area [\(Table 1\)](#page-1-0). Eight 24-mo-old Nellore steers (342 ± 12.0 kg body weight [**BW]**) fitted with ruminal cannulas were used in this study.

The experimental area consisted of 2 ha of Marandu Palisadegrass [*B. brizantha* (Hochst. Ex A. Rich.) Stapf. cv. Marandu) divided into 16 paddocks (1,250  $m^2$  each) and subjected to rotational stocked managements. All paddocks were grazed to a 15-cm post-grazing sward height and fertilized with 40 kg nitrogen·ha−1·grazing cycle−1 during the experiment. In our study, the grazing cycle was considered every time that the pastures reached the target pre-grazing sward height. Thus, the same pastures were grazed multiple times throughout the study.

The experimental area was managed at 25-cm pre-grazing sward height and 15-cm post-grazing sward height during 2009 and 2010, and 4 mo before starting the experimental period, the target pre-grazing sward heights for this experiment (25 and 35 cm) were implemented to consistently achieve treatment differences between sward heights. Sward height measurements were taken every day, for all pastures, as a way to control pasture height. Measurements of sward height (*n* = 20 readings per paddock) were made using a ruler and an acetate sheet in each paddock during every grazing cycle (da Silva et al., 2013).

# **Experimental treatments and management practices**

Treatments consisted of two levels of energy supplementation (0% [none] or 0.3% BW of finely ground corn; mean particle size = 1.3 mm; as-fed basis) and two pre-grazing sward heights (25 or 35 cm). Pre-grazing sward heights, 25 or 35 cm, were randomly assigned to each of the 16 paddocks. Thus, a total of eight paddocks were managed at 25-cm pre-grazing sward height and eight paddocks at 35-cm pre-grazing sward height. Steers within its respective treatment group (25 cm and 35-cm pre-grazing height) grazed together in each paddock. The pregrazing sward heights (25 and 35 cm) were based on research with Marandu Palisadegrass correlating these heights to 95% and ≥97.5% LI, respectively (Giacomini et al., 2009a, 2009b). According to Lemaire and Chapman (1996), the 95% LI represents

<span id="page-1-0"></span>



1 Weather data for the experimental period were recorded at a weather station located 1 km from the experimental area. 2 Average from 1981 to 2010.

the inflection point of the crop growth curve and this reason is the optimal condition for the initiation of grazing on rotationally stocked pastures.

Forage mass and its morphological composition were measured during every grazing cycle before steers started grazing and after they left a paddock. For each measurement, three points were randomly selected in the paddock; at each point, forage within a rectangular frame (0.5 m²) was cut to ground level. Pre-grazing forage mass was calculated and used to adjust forage allowance (6% of BW) for steers grazing paddocks at both sward heights. Such calculation was performed to eliminate forage allowance effects on forage intake, grazing behavior, and pasture occupation period. To accomplish that, extra steers were used to adjust the stocking rate. The occupation period varied according to the respective collection day. For example, grazing behavior was performed in 1 d of the occupation period. As such, the steers started grazing the pasture at its respective treatment height and were removed from the paddock when they reached a 15-cm stubble height. The stocking rate was adjusted to achieve such occupation period.

The experiment lasted 64 d and consisted of four experimental periods of 16 d each. Steers were weighed unfasted prior to the start of each experimental period and the amount of supplement to be fed (kg−1·steer−1·d−1) was fixed for that experimental period. The first 8-d were used to adapt the steers to the experimental treatments and external markers. During the first 8 d, steers in each grazing management treatment grazed the first paddock. The following 8-d consisted of sample collections in the following order: fecal collection from day 9 to 13, animal behavior on day 14, blood and urine collection on day 15, and rumen fluid collection on day 16. On the first day of fecal sample collection, steers were allocated to a new paddock (the second paddock), where they remained for the fecal sample collection (5 d). After that, steers were allocated to a three new paddocks in consecutive days for the animal behavior (third paddock), blood, urine (fourth paddock), and rumen fluid collections (fifth paddock). The number of total paddocks visited for each grazing management (25 or 35 cm) was five per experimental period. Steers had free access to water and mineral supplement (containing 60 g/kg Ca, 20 g/kg P, 72 g/kg Na, 4.2 g/kg S, 1,400 mg/kg of Zn, 400 mg/ kg of Cu, 75 mg/kg of Co, 50 mg/kg of I, and 10 mg/kg of Se) in every paddock for the duration of all the experiment. Steers fed supplement or not were brought daily to a management center close to the experimental area. Supplement was fed in individual feed bunks from 1000 to 1040 hours, and the total amount was recorded. This management center was also utilized for infusing external markers and collecting fecal, blood, urine, and rumen fluid samples.

#### **Forage intake and apparent digestibility**

Forage intake was calculated from total fecal excretion and the digestibility of feeds. On day 1 and for 13 consecutive days, 10 g of chromium oxide (Cr<sub>2</sub>O<sub>3</sub>), previously weighed in two paper capsules (5 g each), was manually dosed into the rumen to all steers, one dose in the morning (1000 hours) and a second dose in the evening (1700 hours). Fecal grab samples (approximately 200 g per steer) were obtained from the rectum of each steer between day 9 and 13, twice a day (1000 and 1700 hours), dried at 55 °C for 72 h, and ground through a 1-mm screen using a Wiley-type mill (MA-680; Marconi Ltda, Piracicaba, SP, Brazil). Equal amounts of dried fecal samples were mixed to obtain one representative sample for each steer for every experimental period. The digestibility of feeds (pasture and supplement) was

estimated using the indigestible neutral detergent fiber (**iNDF**) content as an internal marker as described by Casali et al. (2008) and Donnelly et al. (2018). Indigestible NDF was determined by in situ incubation for 240 h using another set of cannulated steers. Neutral detergent fiber **(NDF**) and crude protein (**CP**) concentrations were analyzed in the feces (Van Soest et al., 1991 and Leco FP-528; LECO Corp., St. Joseph, MI, respectively) and multiplied by fecal production to calculate CP and NDF digestibilities.

For the analyses of fecal chromium concentration, approximately 0.3 g of sample was digested with 6 mL nitric acid and 2 mL perchloric acid and then made up to 30 mL with water (Vega and Poppi, 1997). The digested samples were analyzed using an inductively coupled plasma spectrometer (5110 ICP-OES Agilent; Agilent, Santa Clara, CA).

Forage intake was calculated according to the following equation: forage intake (kg/d) = [total fecal production (kg/d) − fecal output from concentrate (kg/d)]/forage indigestibility (%). Total fecal production was calculated as follows: total fecal production  $(g/d)$  = amount of marker dosed daily  $(g/d)$ / concentration of marker in feces (%). Fecal output coming from the concentrate was calculated according to the following formula: fecal output from concentrate  $(kg/d)$  = concentrate intake (kg/d)/concentrate indigestibility (%).

#### **Grazing behavior**

Grazing behavior measurements took place on day 14 of each period. Four trained observers were assigned to visually determine animal behavior: grazing (eating plus searching), rumination, and resting time, during a period of 24 h. Within this time period, animal behavior was assessed every 5 min by the trained observers, and the information was recorded in a spreadsheet. The total number of observations for each behavioral activity during the 24 h was counted and then multiplied by 5 (i.e. 5-min time interval) to get the daily amount of time spent in each activity. While grazing, cattle search, acquire into the mouth, masticate, and swallow herbage. The observers also counted the bite rate (bites/min) using the time spent for 20 bites (Penning and Rutter, 2004).

Each eating step was considered a feeding station (Ruyle and Dwyer, 1985). The feeding stations per minute were calculated using the time spent at 10 feeding stations divided by 10. The number of steps between feeding stations was determined and the total steps per day were calculated by multiplying the steps per minute by grazing time (min).

#### **Feed sample collection, chemical analysis, and calculations**

Forage mass and its morphological composition were measured during every grazing cycle before steers started grazing and after they left a paddock. For each measurement, three points were randomly selected in the paddock; at each point, forage within a rectangular frame (0.5  $m<sup>2</sup>$ ) was cut to ground level. Total forage mass was weighed and a representative subsample of 0.5 kg was taken and separated into leaf blades, stems (including leaves sheaths), and dead material (as indicated by more than 50% of the tissue area being senescent) to determine the sward morphological composition. After collection, all samples were dried at 55 °C for 72 h. Pre- and post-grazing sward height forage mass and morphological composition are presented in [Table 2.](#page-3-0)

Forage contained in the 15 to 25 cm and in the 15 to 35 cm grazing horizons was collected within a rectangle frame (0.5  $\mathrm{m}^2$ ) at three locations prior to the steers entry into each paddock <span id="page-3-0"></span>**Table 2.** Forage mass and morphological composition of Marandu Palisadegrass (*B. brizantha* [Hochst. Ex A. Rich.] Stapf. cv. Marandu) at two pre-grazing sward heights during the rainy season and measured before and after grazing<sup>1</sup>



1 Measurements of sward height (*n* = 20 readings) were made using a ruler and an acetate sheet in each paddock during every grazing cycle. Forage mass and its morphological composition were measured during every grazing cycle before steers started grazing and after they left a paddock. For each measurement, three points were randomly selected in the paddock; at each point, forage within a rectangular frame (0.5 m²) was cut to ground level.

for every grazing cycle. Each batch of ground corn used as a supplement was sampled. Feed samples were oven-dried at 55 °C for 72 h and ground through a 1-mm screen using a Wiley-type mill (MA-680; Marconi Ltda, Piracicaba, SP, Brazil). Pasture samples were composited by treatment and by period during the experiment. Corn samples were bulked as one composite sample. All samples were analyzed for dry matter ([**DM**] method 930.15; AOAC, [1](#page-8-0)986), ash (method 942.05; AOAC, [1](#page-8-0)986), ash-corrected NDF (Van Soest et al., 1991) using sodium sulfite for all the samples and heat-stable  $\alpha$ -amylase for corn sample, acid detergent fiber (**ADF**) and lignin (Goering and Van Soest, 1970), ether extract (method 920.85; AOAC, [1](#page-8-0)986), and total nitrogen (Leco FP-528; Leco Corp., St. Joseph, MI). A pasture subsample was taken and analyzed for NDF with no sulfite used since nitrogen fractions in the NDF were determined. Neutral detergent insoluble nitrogen (**NDIN**) and acid detergent insoluble nitrogen (**ADIN**) were determined using micro Kjeldahl (AOAC, 1990) after digestion of samples with neutral (no sulfite) and acid detergents. The total digestible nutrients (**TDN**) values of feed samples were calculated according to Weiss et al. (1992). Forage samples were also analyzed for soluble nitrogen (Krishnamoorthy et al., 1982) and for non-protein nitrogen (**NPN**; Licitra et al., 1996) to estimate the protein fractions (Sniffen et al., 1992). The protein fractions were fractions were estimated according to Sniffen et al. (1992): **A** = soluble NPN, **B1** = soluble true protein, **B2** = protein with intermediate rates of degradation, **B3** = insoluble protein in neutral detergent solution but soluble in acid detergent solution, **C** = unavailable nitrogen.

## **Microbial protein synthesis and nitrogen efficiency**

Nitrogen intake (**NI**) was calculated by the sum of NI from forage and supplement. Nitrogen balance was calculated for every steer by dividing mean retained nitrogen (NI minus nitrogen in urine and feces) by the average NI.

The microbial synthesis was estimated through the excretion of purine derivatives (**PD**; Stangassinger et al., 1995). Spot urine samples were collected by spontaneous urination on day 15 of each experimental period in a single sample collected approximately 3 h after supplement feeding. Subsamples of urine (10 mL) were acidified (pH < 3.0) using 40 mL of 0.036 N H<sub>2</sub>SO<sub>4</sub> and stored at −20 °C (Valadares et al., 1999). Urine samples were analyzed for creatinine, allantoin, and uric acid using an HPLC (ShimadzuClass-VP, version 5.03, Shimadzu Scientific Instruments, Inc., Columbia, MD) (Pimpa et al., 2001), and for nitrogen using micro Kjeldahl (AOAC, 1990).

Total urine production was estimated using the creatinine content in the sample, assuming creatinine excretion as 0.213 mmol/kg of BW (Chizzotti et al., 2008). The excretion of PD was then calculated as the sum of allantoin and uric acid excreted in the urine, expressed in mmol/d. The analyses of PD were performed according to Pimpa et al. (2001) using values of the endogenous contribution of PD for zebu cattle, and the intestinal flow of microbial nitrogen compounds was calculated on the basis of microbial purines absorbed as described by Chen and Gomes (1992).

# **Rumen fluid and blood samples**

Rumen fluid was collected to determine ammonia nitrogen (N-NH3 ) and volatile fatty acids (**VFA**) concentrations. On day 16 of every experimental period, rumen fluid was collected at 0, 2, 4, 6, and 8 h after supplement feeding, from three locations in the ventral rumen (approximately 100 mL) by using a probe made with a polyvinyl chloride pipe with small holes in an end to avoid ruminal content being collected and a hose inside the pipe that was connected to a syringe in the other end (Danes et al., 2013). Rumen fluid pH was measured immediately using a portable pH meter (Digimed Model DM22, Digicrom Analítica Ltda., São Paulo, SP, Brazil) and samples were frozen at −20 °C for further analyses. At the end of the experiment, rumen fluid samples were thawed at room temperature, centrifuged (15,000  $\times$  *g*, 4 °C, 30 min), and analyzed for VFA using gas chromatography (Palmquist and Conrad, 1971), and for N-NH<sub>3</sub> by the phenol-hypochlorite procedure (Chaney and Marbach, 1962).

Blood samples were collected on day 15 of the experimental period 3 h after feeding the supplement, from the coccygeal vein of each steer, directly into a lithium heparin-coated vacutainer (Vacuette, Greiner Bio-One, Americana, SP, Brazil). The vacutainers were inverted six to eight times and placed on ice. Plasma was collected after centrifugation (3,000 × *g*, 4 °C, 20 min) and stored frozen at −20 °C for further analyses. Plasma glucose concentration was analyzed by automatic biochemical analyzer YSI Model 2700 Select Biochemistry Analyzer (Yellow Springs Instrument Co. Inc., Yellow Springs, OH).

#### **Statistical analysis**

The experimental design was a replicated  $4 \times 4$  Latin square design, with eight animals, four treatments, and four periods. All variables were analyzed using the MIXED procedure of SAS statistical system (SAS Inst. Inc., Cary, NC). The model included treatment (pre-grazing sward heights [25 or 35 cm], level of energy supplementation [0% or 0.3% BW], and interaction

between pre-grazing sward heights × level of energy supplementation), animal, period, and Latin square as sources of variation to analyze the data of grazing behavior, forage and nutrient intakes and digestibilities, plasma glucose, nitrogen metabolism, and microbial protein synthesis. Treatments and its interaction were considered fixed effects, while Latin square, period, and animal within Latin square were considered random effects.

The model used to analyze rumen parameters (pH and concentrations of VFA and  $\mathrm{NH}_3$ -N) included treatment, time (collection time), and the interaction treatment  $\times$  time as fixed effect, and period, animal within Latin square, and Latin square as random effect. The covariance structure used was Autoregressive order one—AR(1), based on Akaike's Information Criterion ([**AIC**]; Wolfinger, 1993). Means were calculated by least-squares means and adjusted for comparison by Tukey test at 5% probability. Significance was declared at *P* ≤ 0.05 and tendency at 0.05 < *P* < 0.10.

# Results

Effects of pre-grazing sward height  $\times$  energy supplementation interaction were not detected  $(P \ge 0.12)$  in this study, and hence, the effects of pre-grazing sward height and energy supplementation are presented separately in the Discussion section. For all response variables analyzed in this study, the interaction treatment  $\times$  time was not significant ( $P \ge 0.05$ ). Thus, the respective *P-*values were not reported in the tables.

# **Pasture and supplement nutritive value**

In this current study, the morphological and chemical composition of pastures are presented as descriptive data ([Table 2\)](#page-3-0). The observed pre-grazing and post-grazing sward heights were 26-14.6 and 37-17.5 cm, for the targets 25-15 and 35-15 cm, respectively. The forage mass on pre-grazing conditions for 25-cm sward height was 8,036 kg/ha while for 35-cm sward height was 8,900 kg/ha. The total amount of stem on 25- and 35-cm pre-grazing sward height was 1,406 and 1,609 kg/ha while leaf mass was 3,567 and 3,307 kg/ha, respectively. For pastures managed at 25-cm pre-grazing sward height, leaf disappearance was 2,577 kg DM/ha, while at 35 cm, it was 2,520 kg/ha.

<span id="page-4-0"></span>**Table 3.** Nutritive value (% DM) of Marandu Palisadegrass (*B. brizantha* [Hochst. Ex A. Rich.] Stapf. cv. Marandu) grazed at two pre-grazing sward heights during the rainy season and of ground corn supplemented to cattle



1 TDN were estimated according to Weiss et al. (1992). 2 DM content = 90%; Mean particle size = 1.3 mm.

Forage CP, NDF, and TDN values for 25-cm pre-grazing sward height were 13.9%, 58.8%, and 57.3%, and for 35-cm were 11.0%, 63.4% and 54.9%, respectively [\(Table 3\)](#page-4-0). Protein fractions (% of CP) A, B, and C for the 25- and 35-cm pre-grazing management were: fraction A: 27.6% and 26.7%, fraction B: 63.5% and 64.1%, and fraction C: 8.9% and 9.2%, respectively [\(Table 4\)](#page-4-1). Ground corn used as the energy supplement contained 9.3% CP and 87.3% TDN ([Table 3](#page-4-0)).

#### **Grazing behavior and feed intake**

Steers grazing pastures at 25-cm pre-grazing sward height spent less time grazing, spent more time resting, and had greater bite rates (*P* < 0.001) compared with steers grazing pastures at 35-cm pre-grazing sward height ([Table 5](#page-5-0)). The number of feeding stations per minute was greater  $(P = 0.01)$ for steers grazing pastures at 25-cm pre-grazing sward height, but these steers took fewer steps between feeding stations  $(P = 0.01$ ; Table 5). Steps per minute was not affected by the pre-grazing sward height (*P* = 0.92). However, as a consequence of longer time spent grazing at the 35-cm pre-grazing sward height, total steps per day tended to be greater for 35-cm sward height (*P* = 0.09), given the fact that steps per minute did not change  $(P = 0.92)$ . Energy supplementation reduced  $(P = 0.02)$ grazing time but had no effect on the other grazing behavior variables  $(P \ge 0.11)$ .

Steers grazing pastures at 25-cm sward height had greater (*P* ≤ 0.04) forage DM, total DM, digestible DM, NDF, and CP intake than those grazing pastures at 35 cm [\(Table 6\)](#page-5-1). Supplementing cattle with ground corn at 0.3% BW reduced forage dry matter intake (**DMI**; expressed as % of BW and kg/d; *P* ≤ 0.04) and tended to reduce NDF intake  $(P = 0.08)$ , but had no effect on total DM, digestible DM, and

<span id="page-4-1"></span>**Table 4.** Protein and carbohydrate (CHO) fractions of Marandu Palisadegrass [*B. brizantha* (Hochst. Ex A. Rich.) Stapf. cv. Marandu] grazed at two pre-grazing sward heights during the rainy season

	Pre-grazing sward height, cm		
Item	25	35	
Protein fractions <sup>1</sup> , % CP			
A	27.6	26.7	
<b>B1</b>	12.7	7.59	
B <sub>2</sub>	37.8	49.6	
B <sub>3</sub>	13.0	6.85	
C	8.93	9.24	
$CHOs$ fractions <sup>2</sup>			
Total CHOs	75.0	78.7	
$A + B1$ , % total CHOs	10.7	6.91	
B2, % total CHOs	78.4	79.8	
C, % total CHOs	10.9	13.3	

<sup>1</sup>Protein fractions were estimated according to Sniffen et al. (1992). A = soluble NPN, B1= soluble true protein, B2 = protein with intermediate rates of degradation, B3 = insoluble protein in neutral detergent solution but soluble in acid detergent solution, C = unavailable nitrogen.

2 CHO fractions were estimated according to Sniffen et al. (1992): The non-fibrous carbohydrates (**NFC**) were determined by the equation: NFC (%DM) = 100 − (%Neutral Detergent Fiberap + %CP + %Ether Extract + %Ash) which correspond to A + B1 fraction and Neutral Detergent Fiberap is equivalent to the NDF corrected for ash and protein. The B2 fraction was determined by the equation: B2 fraction (%) = %NDF − (%NDIN × 0.01 × %CP) – C fraction. The C fraction was determined by the equation: C fraction  $(\%) = \%NDF \times$  $0.01 \times \%$ Lignin  $\times$  2.4.



<span id="page-5-0"></span>**Table 5.** Effects of pre-grazing sward height and energy supplementation level on grazing behavior of Nellore steers grazing Marandu Palisadegrass (*B. brizantha* [Hochst. Ex A. Rich.] Stapf. cv. Marandu) during the rainy season

1 Supplementation levels were 0% or 0.3% BW of ground corn on an as-fed basis.

2 The experiment lasted 64 d and consisted of four experimental periods of 16 d each. Grazing behavior measurements took place on day 14 of each period. Four trained observers were assigned to visually determine grazing (eating plus searching), rumination, and resting time every 5 min for consecutive 24 h. The number of observations for each activity during a 24-h period was multiplied by 5 min to get the daily amount of time spent in that activity.

<span id="page-5-1"></span>Table 6. Effects of pre-grazing sward height and energy supplementation level on intake of forage DM, total DM (forage + concentrate), digestible DM, NDF, and CP, and substitution rate of forage by supplement of Nellore steers grazing Marandu Palisadegrass [*B. brizantha* (Hochst. Ex A. Rich.) Stapf. cv. Marandu] during the rainy season



1 Supplementation levels were 0 or 0.3% BW of ground corn on an as-fed basis.

2 The experiment lasted 64 d and consisted of four experimental periods of 16 d each. Intakes were calculated from total fecal excretion and the digestibility of feeds. On day 1 and for 13 consecutive days of each period, 10 g of chromium oxide, previously weighed in two paper capsules (5 g each), was manually dosed into the rumen to all steers, one dose in the morning (1000 hours) and a second dose in the evening (1700 hours). Fecal grab samples (approximately 200 g per steer) were manually obtained from the rectum of each steer between days 9 and 13, twice a day (1000 and 1700 hours).

CP intake on either a percentage of BW or kg/day basis (*P* = 0.28; [Table 6\)](#page-5-1). Feeding supplement at 0.3% BW resulted in a substitution rate of 1.47 kg/kg ±0.20 (average ±SD; DM basis).

## **Apparent digestibility**

Forage DM and CP and also diet DM and CP digestibilities were greater ( $P \le 0.05$ ) for steers grazing pastures at 25 cm than for 35-cm pre-grazing sward height [\(Table 7\)](#page-6-0). Energy supplementation had no negative effect on forage (*P* = 0.39) and diet (*P* = 0.77) NDF digestibility but increased (*P* < 0.001) diet DM digestibility ([Table 7\)](#page-6-0).

# **Rumen fluid and plasma glucose**

Steers grazing pastures at 25-cm pre-grazing sward height had lower rumen pH (P < 0.001) and greater rumen N-NH<sub>3</sub> than

cattle grazing pastures at 35 cm (Table 8). Pre-grazing sward height strategy had no effect on the concentration of total VFA, molar proportions of VFA, and plasma glucose  $(P \ge 0.22)$ . Energy supplementation had no effect on rumen pH, total VFA concentration, and on plasma glucose ( $P \ge 0.57$ ) but increased ruminal molar proportion of propionate (*P* < 0.001) and reduced ruminal molar proportion of acetate, acetate:propionate ratio, and rumen N-NH<sub>3</sub> ( $P \le 0.01$ ; [Table 8](#page-6-1))

#### **Microbial protein synthesis and nitrogen utilization**

Energy supplementation did not increase nitrogen utilization (% of NI), microbial synthesis, and efficiency (*P* ≥ 0.36; [Table 9\)](#page-7-0). However, steers grazing pastures at 25-cm pre-grazing sward height had greater NI and retention  $(P \le 0.04;$  [Table 9\)](#page-7-0). Microbial nitrogen efficiency tended to be greater for steers

<span id="page-6-0"></span>**Table 7.** Effects of pre-grazing sward height and energy supplementation level on total tract digestibility of DM, CP, and NDF of the forage and total DM (forage + supplement) of Nellore steers grazing Marandu Palisadegrass (*B. brizantha* [Hochst. Ex A. Rich.] Stapf. cv. Marandu) during the rainy season

Item $2$	Pre-grazing sward height, cm		Supplememntation <sup>1</sup> , %BW			P-value		
	25	35	$\mathbf 0$	0.3	<b>SEM</b>	Sward height	Supplementation	Interaction
	Forage digestibility, %							
DM	68.1	65.4	67.3	66.2	0.78	< 0.001	0.16	0.65
CP	74.6	63.8	70.1	68.3	0.94	< 0.001	0.18	0.85
<b>NDF</b>	63.4	62.5	62.4	63.5	1.19	0.51	0.39	0.55
Diet digestibility, %								
DM	70.1	68.4	67.3	71.2	0.97	0.05	< 0.001	0.12
CP	75.8	66.5	70.1	72.2	1.36	< 0.001	0.13	0.17
<b>NDF</b>	63.1	62.1	62.4	62.8	1.16	0.44	0.77	0.63

1 Supplementation levels were 0% or 0.3% BW of ground corn on an as-fed basis.

2 The experiment lasted 64 d and consisted of four experimental periods of 16 d each. On day 1 and for 13 consecutive days of each period, 10 g of chromium oxide, previously weighed in two paper capsules (5 g each), was manually dosed into the rumen to all steers, one dose in the morning (1000 hours) and a second dose in the evening (1700 hours). Fecal grab samples (approximately 200 g per steer) were manually obtained from the rectum of each steer between days 9 and 13, twice a day (1000 and 1700 hours). The digestibility of feeds (pasture and supplement) was estimated using the iNDF content as an internal marker as described by Casali et al. (2008).

<span id="page-6-1"></span>**Table 8.** Effects of pre-grazing sward height and energy supplementation level on rumen fluid pH, N-NH<sub>3</sub>,VFA, and plasma glucose concentration of Nellore steers grazing Marandu Palisadegrass (*B. brizantha* [Hochst. Ex A. Rich.] Stapf. cv. Marandu) during the rainy season



1 Supplementation levels were 0% or 0.3% BW of ground corn on an as-fed basis.

2 The experiment lasted 64 d and consisted of four experimental periods of 16 d each. Rumen fluid (approximately 100 mL) was collected at 0, 2, 4, 6, and 8 h after supplement feeding with the use of a sampling probe (Danes et al., 2013) on day 16. Blood samples were collected on day 15 of the experimental period 3 h after feeding the supplement, from the coccygeal vein of each steer, directly into a lithium heparin-coated vacutainer.

grazing pastures at 35-cm pre-grazing sward height (*P* = 0.09; [Table 9\)](#page-7-0).

# **Discussion**

#### **Pre-grazing sward height**

When pastures are managed based on the 95% LI as start grazing point criteria, the forage offered to the animal is of better quality (Pedreira et al., 2017) and the sward structure allows greater grazing efficiency (da Silva et al., 2013).

In the current study, using 95% LI to start grazing point criteria resulted in a 30% greater forage intake and 28% greater intake of digestible DM in a shorter grazing time period. Stems may serve as a horizontal barrier and reduce bite area, bite rate, and consequently forage intake (Benvenutti et al., 2009; da Silva et al., 2013). Hodgson et al. (1977) pointed out that sward structure may have a more negative impact on forage intake of

grazing cattle than rumen fill because of the limitations imposed on the harvesting process. The greater DM and digestible DM intakes observed for steers grazing 25-cm pre-grazing sward height pastures can be explained by the greater proportion of green leaves, lower proportion of dead material (Hodgson et al., 1977), lower NDF content, and greater DM digestibility (Conrad et al., 1964) found in 25-cm pre-grazing pastures.

In pastures with greater sward height, cattle present greater bite mass (Carrère et al. (2001) and spend more time manipulating and chewing (Benvenutti et al., 2009; da Silva et al., 2013), which results in more time spent for each bite interval and subsequently reduces bite rate as reported in the present study.

A feeding station is defined as the area of pasture a grazing animal can reach at each eating step (Searle et al., 2005). The forage allowance at each feeding station is associated with the time spent by the animal at the feeding station (Carrère et al., 2001). Animals start grazing at one feed station, staying there



<span id="page-7-0"></span>**Table 9.** Effects of pre-grazing sward height and energy supplementation level on nitrogen metabolism of Nellore steers grazing Marandu Palisadegrass (*B. brizantha* [Hochst. Ex A. Rich.] Stapf. cv. Marandu) during the rainy season

1 Supplementation levels were 0% or 0.3% BW of ground corn on an as-fed basis.

2 The experiment lasted 64 d and consisted of four experimental periods of 16 d each. Spot urine samples were collected by spontaneous

urination on day 15 of each experimental period in a single sample collected approximately 3 h after supplement feeding.

3 MicEf = microbial efficiency: g of microbial CP/kg TDN. Microbial synthesis was estimated through the excretion of PD (Stangassinger et al., 1995).

until the forage allowance is reduced before choosing another feeding station (Searle et al., 2005). The distance walked between feeding stations may indicate how difficult is to find another new feeding station (Utsumi et al., 2009). Steers grazing pastures managed at 35-cm pre-grazing sward height spent more time per feeding station, resulting in fewer feeding stations visited per minute, which is related to increased forage mass in this study, greater amount of forage per bite, and greater time spent manipulating it before swallowing at each feeding station (Carrère et al., 2001; Utsumi et al., 2009). The greater number of steps between feeding station and the total steps taken per day in steers at 35-cm pre-grazing sward height indicated that animals walked more searching for better feeding stations.

The greater intakes of forage and of digestible DM and the greater resting time (suggesting less energy expenditure) of cattle grazing Palisadegrass pasture managed at 25-cm height might result in greater cattle average daily gain (**ADG**). Gimenes et al. (2011) reported greater ADG (0.63 and 0.51 kg/d) and stocking rate (3.13 and 2.85 AU/ha) for cattle grazing Palisadegrass managed at 25- vs. 35-cm pre-grazing sward height. Giacomini et al. (2009b) reported that Marandu Palisadegrass pastures managed with 95% LI at pre-grazing and 15-cm post-grazing height presented greater crop growth rate than pastures managed with 100% LI. Grazing efficiency is also greater when cattle graze pastures at 95% LI (Gomes et al., 2018). Both, greater forage growth rate and grazing efficiency support the greater stocking rate reported by Gimenes et al. (2011).

Ruminal pH was lower in cattle at 25-cm compared with 35-cm pre-grazing sward height probably because of the greater amount of feed being fermented in the rumen of cattle that consumed more DM with greater digestibility. Palisadegrass managed at 25-cm pre-grazing canopy height presents earlier stages of maturity and greater leaf:stem ratio compared with 35-cm or higher sward height (Gimenes et al., 2011; Pedreira et al., 2017; Gomes et al., 2018). These conditions benefit the fiber digestion in the rumen due to more favorable morphological and anatomical characteristics of the forage (Akin et al., 1987).

The greater CP content and degradability (A + B1 fractions) of pastures managed at 25-cm pre-grazing sward height associated with the greater DMI and consequent greater N intake compared with 35-cm pre-grazing sward height can explain the greater ruminal N-NH<sub>3</sub> observed in the current trial. However, the levels of ruminal N-NH<sub>3</sub> in both grazing management did not limit microbial growth (Satter and Slyter, 1974) neither NDF

digestibility (Sampaio et al., 2010). Sampaio et al. (2010) reported that rumen  $N\text{-}NH_{_3}$  concentration of 6.24 mg/dL required to maximize NDF degradation of tropical grasses.

Improving pasture management was an effective strategy to increase the efficiency of N utilization. Cattle grazing the 25-cm sward height pasture consumed more N, excreted the same amount of N, and hence retained more N than cattle grazing the 35-cm pasture. Cattle grazing the 25-cm pasture consumed 27% more digestible DM and 67% more CP compared with steers grazing 35-cm pastures. The greater nitrogen retention in steers grazing 25-cm pastures was likely caused by higher intakes of digestible DM compared with steers grazing 35-cm pastures and both facts support the greater ADG of cattle grazing pastures with 25 cm compared with 35 cm as reported by Gimenes et al. (2011).

#### **Energy supplementation**

The main goal when using energy supplementation is to increase energy intake and performance of grazing cattle (Cappellozza et al., 2014; Silva et al., 2018). In the present study, supplementing energy at 0.3% BW caused substitution effect great enough to prevent an increase in energy intake by cattle grazing tropical pastures. When energy intake is not increased by supplementation, animal gain is also not altered. In pastures subjected to rotational stocked managements, the forage allowance and the leaf:stem ratio decrease dramatically during the grazing down process. Under this condition, it might be speculated that a small amount of energy supplement is able to cause a great substitution rate which has not been reported in continuous grazing studies (Barbero et al., 2015; de Oliveira et al., 2015).

It has been reported in some studies that energy supplementation can reduce rumen pH and fiber digestibility, resulting in lower forage intake (Leventini et al., 1990). However, in the present study, corn supplementation reduced forage DMI but it did not cause any reduction in rumen pH and in NDF digestibility, as reported by others (Chase and Hibberd, 1987; Pordomingo et al., 1991; Elizalde et al., 1998). This finding is similar to other studies (Lake et al., 1974; Branine and Gaylean, 1995; Caton and Dhuyvetter, 1997), which reported either an improvement or no effect of energy supplementation on DM, OM, or NDF digestibility for cattle consuming forage diets. According to Caton and Dhuyvetter (1997), at lower levels of supplementation, responses in fiber digestibility and rumen pH

are much less predictable and the data of studies published in the literature clearly suggest that responses are not consistent. Additionally, substitution rates seem to be responsive to forage quality, as demonstrated by Minson (1990) who reported that as forage CP increases, substitution coefficients increase. Highquality forage combined to low levels of supplementation may potentially be one of the main factors creating inconsistent results in research studies evaluating energy supplementation for grazing cattle.

Considering that corn can provide a potential substrate for propionate production, some authors (Martin and Hibberd, 1990; Faulkner et al., [199](#page-9-1)4) attributed increases in propionate concentration and changes in acetate:propionate ratio to supplementation. Similar observations were made in this study when 0.3% BW of corn was fed and it decreased molar proportion of rumen acetate, increased rumen propionate, and decreased the acetate:propionate ratio. However, plasma glucose was not increased by feeding corn to grazing cattle.

For both pasture treatments, either the CP:TDN ratio or the high  $A + B1$  protein fraction and the low  $A + B1$  CHO fraction indicate an imbalance between energy and protein in forage and may limit the efficiency of protein utilization (Poppi and McLennan, 1995). According to these authors, supplementing rumen fermentable energy may be an effective tool to improve the performance of grazing cattle and the utilization of forage protein. However, despite the decrease in rumen N-NH3, corn supplementation did not increase N retention and microbial yield. The low CP content from corn (energy supplement) combined with the reduction in forage intake decreased protein content of the total diet of supplemented animals, which resulted in a lower concentration of rumen N-NH<sub>3</sub>. The low levels of CP observed in pastures managed at 35 cm associated with low levels of forage intake resulted in a lower concentration of rumen N-NH $_{\textrm{\tiny{\textup{3}}}}$  compared with steers grazing pastures managed at 25 cm. Grazing management caused a reduction in rumen N-NH $_{\rm_3}$  concentration to a level that was similar to the main effect of energy-supplemented treatment. Additionally, the decrease in rumen N-NH $_{\rm_3}$  when corn was fed may be related to the lower degradability of corn protein (NRC, 1996) compared with protein from Palisadegras and less protein in the diet. Our findings highlight the importance of grazing management as an important tool to increase nutrient intake and efficiency of nutrient usage.

Satter and Slyter (1974) reported that concentrations between 2 and 5 mg N-NH $_{\textrm{s}}$ /dL of rumen fluid were required to allow maximum microbial growth. Sampaio et al. (2010) reported 6.24 mg/dL to maximize NDF degradation in animals consuming tropical grass (*Brachiaria decumbens*). In the current study, supplemented and non-supplemented steers had rumen concentrations of N-NH $_{\rm_3}$  above the minimum values required to optimize microbial yield and fiber degradation (Satter and Slyter, 1974; Sampaio et al., 2010).

Studies indicate that microbial synthesis is maximized when rumen fermentable CHOs and degradable proteins are available (Russell et al., 1992; Bach et al., 2005). An increase in microbial synthesis was expected with energy supplementation because rumen microbes would better utilize forage nitrogen due to a greater quantity of readily fermentable CHOs in the rumen (Russell et al., 1992; Owens and Goetsch, 1993). However, the high substitution rate resulted in a similar intake of digestible DM between supplemented and non-supplemented cattle. Since corn supplementation did not alter N and digestible DM intakes of grazing cattle, it had no effect on N excretion and on N retention.

# **Conclusions**

When cattle graze Marandu Palisadegrass at 25 cm and 95% LI, they consume more digestible DM and retain more N with less grazing time, compared with 35-cm pre-grazing sward heights. Such findings are in agreement with the first hypothesis of this study.

Contrary to the second hypothesis, feeding energy supplement at 0.3% BW does not increase digestible DM intake and N retention of cattle grazing Marandu Palisadegrass due to a high substitution rate of 1.4 kg reduced forage intake per kg supplement. Feeding higher levels of energy supplementation for cattle rotationally grazing pastures deserves further evaluation as an attempt to increase animal energy intake and N retention or increase stocking rates.

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## Conflict of interest statement

The authors declare no conflicts of interest.

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