

**University of São Paulo  
“Luiz de Queiroz” College of Agriculture**

**Energy supplementation for beef steers grazing tropical grass  
(*Brachiaria brizantha*, cv Marandu) managed under rotational  
system with different initial sward heights**

**João Ricardo Rebouças Dórea**

Thesis presented to obtain the degree of Doctor in  
Science. Area: Animal Science and Pastures

**Piracicaba  
2014**

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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## RESUMO

### **Suplementação energética para bovinos mantidos em pastagem tropical (*Brachiaria brizantha*, cv Marandu) manejados em sistema de pastejo rotativo com diferentes alturas de entrada**

Dois experimentos foram conduzidos simultaneamente, para avaliar o uso da suplementação energética para bovinos manejados em diferentes alturas de entrada na pastagem. Foram usados 8 novilhos Nelore canulados no rumen por experimento (Exp. 1: 300 kg de PC  $\pm$  5,97, Exp. 2: 343 kg PC  $\pm$  7,40) distribuídos em 2 quadrados latinos 4x4. Os tratamentos para o Exp. 1 foram 0 (suplementação mineral) e 0,3 (0,3% do PC em milho moído) combinados com 2 alturas de entrada (25 e 35 cm). A altura de saída foi 15 cm. No Exp. 2 o nível de suplementação foi 0,6% do PC em milho moído. Os animais foram manejados em 2 ha de Capim Marandu, os quais foram adubados com 120 kg de N/há, apresentando valores médios de 13,8 e 11,0% de PB e 58,8 e 63,4% de FDN para pastos de 25 and 35 cm, respectivamente. A DMS e DPB da forragem e da dieta foram maiores ( $P < 0,05$ ) para o manejo da pastagem de 25 cm do que 35. Em ambos os experimentos, o CMS de forragem, energia e total foi maior ( $P < 0,05$ ) para o tratamento de 25 cm, que ao mesmo tempo promoveu menor tempo de pastejo ( $P < 0,05$ ), maior tempo em ócio ( $P < 0,05$ ) e taxa de bocado ( $P < 0,05$ ), menor número de passos por dia e passos entre estações de pastejo ( $P < 0,05$ ), quando comparados com animais mantidos no tratamento de 35 cm. O pH ruminal foi menor ( $P < 0,05$  no Exp. 1;  $P < 0,10$  no Exp. 2), a N-NH<sub>3</sub> ruminal e retenção do N foram maiores ( $P < 0,05$ ) para animais manejados na altura de entrada de 25 cm. Os AGVs e a síntese microbiana não foram afetados ( $P > 0,05$ ) pelo manejo da pastagem. A suplementação em 0,3% (Exp. 1) aumentou ( $P < 0,05$ ) a DMS da dieta, enquanto a suplementação de 0,6% (Exp. 2) reduziu a DPB da forragem ( $P < 0,05$ ), aumentou a digestibilidade da FDN da forragem ( $P < 0,05$ ) e a DMS ( $P < 0,05$ ) e da FDN da dieta ( $P < 0,01$ ). A suplementação em 0,3% (Exp. 1) ou em 0,6% (Exp. 2) reduziu o CMS de forragem ( $P < 0,05$ ) e as taxas de substituição foram 1.63 and 0.72, respectivamente. O CMS total e de energia não foram aumentados ( $P > 0,05$ ) pela suplementação em 0,3%, enquanto o nível de 0,6% foi efetivo em aumentar o CMS total e de energia de bovinos mantidos em pastagem tropical, independente do manejo da pastagem. A suplementação reduziu o tempo de pastejo ( $P < 0,05$ ). Animais suplementados com 0,3% não alteraram ( $P > 0,05$ ) o pH ruminal, a retenção de N e síntese microbia, mas aumentaram ( $P < 0,05$ ) propionato no rumen e diminuíram ( $P < 0,05$ ) N-NH<sub>3</sub> ruminal, acetato e relação acetato:propionato. A suplementação com 0,6% diminuiu ( $P < 0,05$ ) o pH ruminal, N-NH<sub>3</sub> ruminal, acetato e relação acetato:propionato no rumen, aumentaram ( $P < 0,05$ ) o propionato no rumen, a retenção de N e a síntese microbiana. A glicose plasmática não foi alterada ( $P > 0,05$ ). A altura de pré-pastejo de 25 cm e a suplementação energética de 0,6% do PC foram estratégias eficientes para aumentar o consumo de energia de bovinos mantidos em pastagens de Capim Marandu.

Palavras-chave: Energia; Forragem tropical; Gado de corte; Manejo da pastagem; Suplementação



## ABSTRACT

### **Energy supplementation for beef steers grazing tropical grass (*Brachiaria brizantha*, cv Marandu) managed under rotational system with different initial sward heights**

Two trials were conducted simultaneously to evaluate the effects of energy supplementation for cattle grazing tropical pastures managed with different initial sward heights on DMI and ruminal fermentation of cattle grazing intensively managed tropical grass during the rainy season. Eight 24-month-old rumen-cannulated Nelore steers were used per trial (Trial 1: 300 kg BW  $\pm$  5.97, Trial 2: 343 kg BW  $\pm$  7.40) allocated in two 4x4 Latin squares. Treatments corresponded to 0 (mineral supplementation) and a 0.3 (0.3% of BW of ground corn as fed basis) combined with 2 pre-grazing sward heights (25 and 35 cm). The stubble height was 15 cm. In the second trial the level of supplementation was 0.6% BW of ground corn as fed basis. Steers were managed in 2 ha of Palisadegrass pasture (*Brachiaria brizantha marandu*). Pastures were fertilized with 120 kg nitrogen/ha and averaged 13.8 and 11.0% CP and 58.8 and 63.4% NDF, for 25 and 35 cm, respectively. The forage and the diet DM and CP digestibility were greater ( $P < 0.05$ ) for 25 than for 35 cm grazing management. For both trials 1 and 2, cattle grazing the pastures with 25 cm initial sward height consumed more forage DMI, more total DMI and more energy ( $P < 0.05$ ) and at the same time steers spent less time grazing ( $P < 0.05$ ) and more time resting ( $P < 0.05$ ), presented greater bite rates ( $P < 0.05$ ), less steps per day and less steps between feeding stations ( $P < 0.05$ ), when compared with cattle grazing the 35 cm pastures. Rumen pH values were less ( $P < 0.05$  in trial 1;  $P < 0.1$  in trial 2) and concentrations of rumen N-NH<sub>3</sub> and retention of N were greater ( $P < 0.05$ ) for cattle grazing the 25 cm pastures while rumen VFA and microbial synthesis were not affected ( $P > 0.05$ ) by pasture management. Supplementing energy at 0.3% (trial 1) increased ( $P < 0.05$ ) diet DM digestibility while feeding energy at 0.6% (trial 2) decreased forage CP digestibility, increased ( $P < 0.05$ ) forage NDF digestibility and increased diet DM ( $P < 0.05$ ) and diet NDF ( $P < 0.1$ ) digestibility. Supplementing energy at 0.3% (trial 1) or at 0.6% (trial 2) decreased forage DMI ( $P < 0.05$ ) and substitution rates were 1.63 and 0.72, respectively. The total DMI and energy intake were not increased ( $P > 0.05$ ) by supplementing energy at 0.3% while increasing energy supplementation to 0.6% was effective to increase total DMI and energy intake of cattle grazing tropical forage, independent of initial sward height. Energy supplementation decreased ( $P < 0.05$ ) grazing time, but it did not affect ( $P > 0.05$ ) any other grazing behavior parameter. Supplementing grazing cattle with 0.3% had no effect ( $P > 0.05$ ) on rumen pH, N retention and microbial synthesis, increased ( $P < 0.05$ ) rumen propionate and decreased ( $P < 0.05$ ) rumen N-NH<sub>3</sub>, rumen acetate and acetate:propionate ratio. Supplementing grazing cattle with 0.6% decreased ( $P < 0.05$ ) rumen pH, rumen N-NH<sub>3</sub>, rumen acetate and acetate:propionate ratio, while it increased ( $P < 0.05$ ) rumen propionate, N retention and microbial synthesis. Plasma glucose was not affected by treatments ( $P > 0.05$ ). The pre-grazing sward height of 25 cm and feeding energy supplement at 0.6% of BW were efficient strategies to increase energy intake of cattle grazing Palisadegrass.

Keywords: Beef cattle; Energy; Grazing management; Supplementation; Tropical grass



## 1 INTRODUCTION

About 92% of Brazilian beef production comes from grazing systems (MILLEN et al., 2009) and the great majority of these grassland areas are covered with tropical grasses. Even in intensive tropical grazing systems based on well managed pastures, cattle ADG and milk production are far lower than animals genetic potential (SANTOS et al., 2014). Most of this limitation comes from limited energy intake, due to limitations in forage harvest capacity of the animals (SANTOS et al., 2009; DANÉS et al., 2013) and rumen fill caused by fiber content and low particle fragility (ALLEN, 2000) of grasses.

There is no doubt on the importance of the physical mechanisms regulating forage intake of cattle. However, for grazing cattle, before the forage can reach the gastrointestinal tract, it has to be harvested by the animal. Hodgson et al. (1977) reported that the structure of the sward may have a greater impact on forage intake of grazing cattle than the physiological mechanisms discussed by Conrad et al (1964) and Allen (2000). The structure of the sward has a great impact on the efficiency of forage harvesting by grazing cattle, either for temperate grasses (HODGSON et al., 1994) as for tropical grasses (DA SILVA; PEDREIRA, 1996; CARVALHO et al., 2001; DA SILVA; CARVALHO, 2005; DA SILVA et al., 2009; PAULA et al., 2012).

The nitrogen fertilization increases the CP content in tropical grasses (JONHSON et al., 2001; DANÉS et al., 2013) which may be adequate or even excessive for growing cattle (NATIONAL RESEARCH COUNCIL - NRC, 1996). However, their sward structure often limits forage harvesting (BENVENUTTI et al., 2006) and its association with high NDF content ( $\geq 50\%$ ) (DANÉS et al., 2013) and low particle fragility (ALLEN, 2000) causing rumen fill, limits forage intake and results in energy being the most limiting nutrient. Thus, supplementing grain or by-products high in energy is a strategy to overcome this limitation. However, energy supplementation may decrease forage intake due to substitution effect (ELIZALDE et al., 1998). This may not be related to rumen pH and its effects on fiber degradation (CATON; DHUYVETTER, 1997). When substitution rate is high, total DMI and energy intake may not be increased, resulting in no improvements in animal performance. Dórea (2011) reported that supplementing ground corn at 0.3% BW for Nelore steers on Palisadegrass cv. Marandú pasture managed under rotational grazing with 25 cm canopy height (corresponding to 95% light interception) (TRINDADE, 2007) as the

start grazing point, did not increase cattle energy intake primarily due to a high substitution rate. When supplement was fed at 0.6% BW, substitution rate decreased and energy intake was increased. Correia (2006) reported a linear increase in cattle ADG to energy supplementation (0.3 vs 0.6 vs 0.9% BW) on Palisadegrass cv. Marandu pasture managed under rotational grazing with high stocking rates during the rainy season. However, the response to the 0.3% BW treatment was quite small. Feeding 0.6% BW resulted in extra ADG 3 times greater than 0.3%BW compared to the no supplemented cattle.

Therefore, practices that improve energy intake by grazing cattle as refinement in pasture management practices and energy supplementation may be powerful tools to improve animal performance and productivity in tropical grazing systems. However, the response to association of these two practices has not been reported in the literature for cattle grazing tropical grasses.

In this context, the hypotheses of this study were: 1) Energy supplement fed at 0.3% BW may cause high substitution rate and consequent no increase in energy intake for cattle on tropical pastures managed under rotational grazing; 2) Feeding an energy supplement at levels greater than 0.3% BW may be required to increase energy intake of cattle on tropical pastures managed under rotational grazing; 3) Interactions may occur between energy supplementation and pasture management practices; 4) the adoption of an ideal start grazing point based on canopy height (correlated to the 95% light interception) may increase forage harvesting efficiency, nutrient intake and metabolism of cattle grazing Palisadegrass cv. Marandu.

The propose of these studies were to evaluate the effects of energy supplementation and its interaction with grazing management on cattle: 1) ingestive behavior; 2) nutrient intake; 3) rumen parameters; 4) blood glucose; 5) apparent digestibility; 6) rumen fermentation kinetics; and 7) nitrogen use.

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## 2 LITERATURE REVIEW

### 2.1 Grazing managements

The structure of the sward has a great impact on the efficiency of forage harvesting by grazing cattle, either for temperate grasses (HODGSON et al., 1994) as for tropical grasses (DA SILVA; PEDREIRA, 1996; CARVALHO et al., 2001; DA SILVA, 2009).

Just after the grazing process, the grass initiates its regrowth with intense leaf appearance and leaf growth and minimal stem elongation. The sward will reach a determined height where light will start to become limiting for the lower portion of the sward. Light restriction will stimulate leaves senescence and stem elongation. The result is an increased proportion of senescent material and stems and a decreased proportion of green leaves in the sward, with dramatic alteration on the grazing efficiency and forage intake (DÓREA et al., 2013a, 2013b).

Rotational grazing based on fixed or pre-established number of days for grazing intervals has been criticized by Da Silva and Corsi (2003). Grass dry matter production is dictated by its genetic potential and by environmental conditions such as temperature, sun light, soil fertility and water availability. As these conditions vary, grazing interval must vary too. The ideal start grazing point should be based on plant physiology aspects instead of on fixed grazing intervals.

The concept of an ideal start grazing point originated from studies with temperate grasses (HODGSON, 1990) using 95% light interception (LI) criterion has been successfully applied to tropical grasses (DA SILVA; NASCIMENTO JÚNIOR, 2007). The latter authors reported a number of studies conducted in Brazil with various tropical grasses and a range of sward height values correlated to 95% LI. The studies reported by Da Silva and Nascimento Júnior (2007) indicated that the sward heights correlating to 95% LI vary with time of the year, but a much higher degree of variation is between plant spp. Some of the work reviewed correlating 95% LI to sward height are presented in Table 1.

Table 1 - Suggested sward and stubble heights for pastures managed based on 95% light interception (LI)

Plant spp.	Sward height (cm)		Reference
	Entrance	Exit	
Mombaça	90	30 to 50	Carnevalliet al., (2006)
Tanzânia	70	30 to 50	Difante, (2005); Barbosa et al., (2007)
Xaraés	30	15 to 20	Pedreira, (2006)
Cameroon	100	40 to 50	Voltolini, (2006); Carareto, (2007)
Marandu	25	10 to 15	Zeferino, (2006); Sarmiento, (2007); Costa (2007); Souza Júnior, (2007); Trindade, (2007)
Tifton-85	25	10 to 15	Da Silva and Corsi (2003)
Coastcross	30	10 to 15	Da Silva and Corsi (2003)

Mombaça = *Panicum maximum* cv. Mombaça; Tanzânia = *Panicum maximum* cv. Tanzânia; Xaraés = *Brachiaria brizantha* cv. Xaraés; Cameroon = *Pennisetum purpureum* cv. Cameroon; Marandu = *Brachiaria brizantha* cv. Marandu; Tifton-85 = *Cynodon dactylon* cv. Tifton-85; Coastcross = *Cynodon dactylon* cv. Coastcross

Morphological composition of plants managed based on 95% light interception (LI) criterion as the ideal start grazing point present significantly higher proportion of leaves and lower proportion of stem and senescent material in comparison to plants managed with fixed grazing intervals (Table 2).

Table 2 - Morphological composition of plants managed either based on 95% light interception (LI) criterion or on fixed grazing intervals

Reference	Forage	Grazing frequency	Leaves % DM	Stem % DM	Senescent material % DM
Voltolini (2006)	Cameroon	27 fixed days	48.0	46.0	6.0
Voltolini (2006)	Cameroon	95% LI	53.0	42.0	5.0
Correia (2006)	Marandu	21 fixed days	34	32.7	33.3
Costa (2007)	Marandu	95% LI	56	32	12

Cameroon = *Pennisetum purpureum* cv. Cameroon; Marandu = *Brachiaria brizantha* cv. Marandu

Carvalho et al. (2001), pointed out that the structure of the sward would have a greater importance in forage intake regulation for grazing cattle than the physiological and chemical mechanisms discussed by Conrad et al. (1964) and Allen (2000) due to its impact on the harvesting process of forage. The impact of the sward structure on forage intake of grazing cattle has been reported by several other authors (DA

SILVA; CARVALHO, 2005; CASAGRANDE, 2010; VIEIRA, 2011; PAULA et al., 2012).

## 2.2 Effects of sward structure on forage intake

Sward structure can be described in terms of forage mass, pasture height, ground cover, and forage density for different strata (CANGIANO et al., 2002). Variations in any of these variables may affect bite density, bite area and bite mass (HODGSON, 1990).

Considering that daily forage intake can be defined as the product of bite mass, bite rate, and grazing time (Alden and Whittaker 1970; Hodgson 1981), then the sward structure can alter significantly the forage intake, and in the most cases for animals in grazing systems can affect the consume more than physical and physiological mechanisms (HODGSON, 1977; CANGIANO et al., 2002).

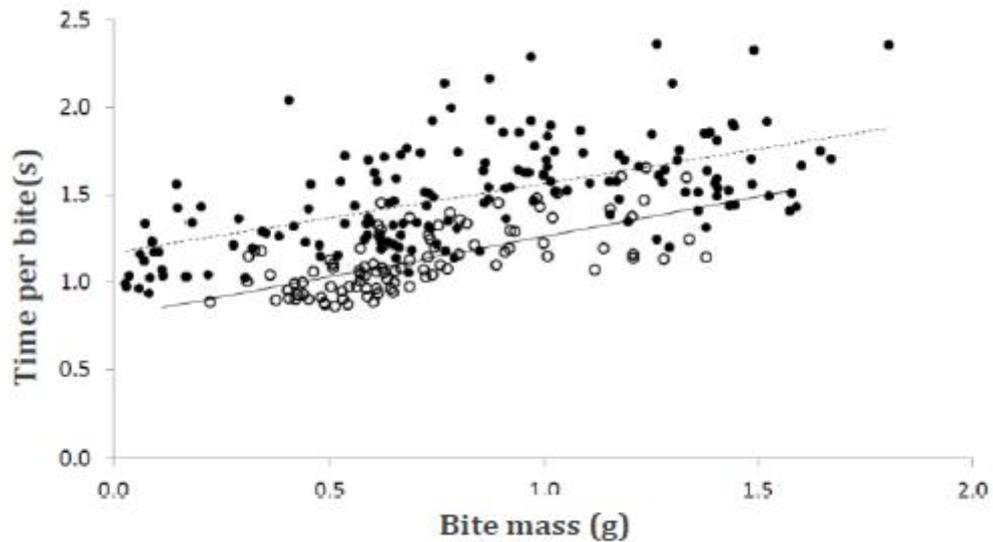
Forage intake is a function of body weight ( $BW^{0.75}$ ) in growing animals, but under grazing conditions intake may be limited by sward characteristics (CANGIANO et al., 2002). Illius and Gordon (1987) described a model in which the allometric relationship between BW and bite area changes with sward height. On short swards, where only a narrow band of tillers can be prehended, bite area is determined by incisor arcade breadth ( $BW^{0.36}$ ). In tall swards, where the maximum bite area can be achieved, bite area is related to the square of the incisor arcade breadth, so it scales to  $BW^{0.72}$ .

To explain and quantify forage intake, a many mechanisms regarding the dynamics of the plant–animal interface must be detailed and understood. Because increases in bite rate or grazing time generally do not compensate for reductions in bite mass, it is the most important variable in daily intake (CHACON; STOBBS, 1976). Bite mass is the product of bite area, bite depth, and pasture bulk density in the grazing horizon (BURLISON; ILLUS, 1991).

There is a historical idea that lower animal production in tropical pastures would be related with low forage quality, as reported by Sollenberger and Burns (2001), where the authors pointed out that tropical pastures produce low-quality forage with high bulk density of pseudostems, and just would support low levels of animal performance.

However, several authors (DA SILVA; PEDREIRA, 1996; CARVALHO et al., 2001; DA SILVA; CARVALHO, 2005; DA SILVA et al., 2009; PAULA et al., 2012) have worked for many years to support an opposite idea, where these authors discussed and concluded that pasture structure was more important in constraining forage intake than previously supposed. In fact, basing pasture management on degree of sward light interception and avoiding stem development has supported new management strategies (TRINDADE, 2007; GIMENES et al., 2011; DANÉS et al., 2013), resulting in unexpected high levels of animal production (CARARETO, 2007; VOLTOLINI et al., 2010; GIMENES et al., 2011).

In the important and recent meta-analysis published by Carvalho (2013) was demonstrated how tropical pasture structure influences forage intake. Carvalho (2013) suggests that grazing animals take more time to gather a given bite mass in tropical than in temperate pastures. In the graphic 1 (CARVALHO, 2013), is possible to observe the intercept of the model refers to the time to prehend the bite, independently of bite mass, what indicate that mistakes on grazing managements in tropical grass may affect drastically DMI when compared with temperate grasses.



Graphic 1 - Temperate pastures (○, solid line): 1 – *Lolium multiflorum* (Amaral et al. 2013); 2 – *Avena strigosa* sward under continuous, and 3 – rotational stocking; 4 – *Lolium multiflorum*, *Avena strigosa* and avena + ryegrass mixture (G.C. Guzatti comm.). Tropical pastures (●, dotted line): 5 – *Cynodon* sp. under rotational, and 6 – continuous stocking; 7 – *Sorghum bicolor* under rotational, and 8 – continuous stocking; 9 – *Brachiaria brizantha* under rotational stocking ; 10 – natural grassland under continuous stocking ; 11 – *Pennisetum glaucum* under rotational stocking. Regression equations have been generated for each species in each experiment, and then compared by parallelism test and equality of intercepts ( $P < 0.05$ ). There are no differences between stocking methods in each group of pastures. Temperate pastures model:  $y = 0.457x + 0.800$ ;  $R^2 = 0.724$ ;  $P < 0.0001$ ; s.e. = 0.142;  $n = 98$ . Tropical pastures model:  $y = 0.395x + 1.166$ ;  $R^2 = 0.489$ ;  $P < 0.0001$ ; s.e. = 0.239;  $n = 185$

Source: Carvalho (2013)

There are many references in the literature showing high contents of NDF in tropical forages (MORAIS et al., 2009; SALES et al., 2009; PORTO et al., 2009) , what associated with inadequate management can intensify troubles on forage intake.

According to Hodgson et al. (1994), the forage quality is usually higher in leaves than in stems and it is generally accepted that animals select leaves and avoid stems. However, recent studies on artificial swards have shown that this selective behavior varies according to the tensile resistance of the stems (BENVENUTTI et al., 2006, 2008). The animals did not select against stem with low tensile resistance but avoided stems of high tensile resistance. As a result, diet quality increased in relation to the quality of the forage on offer but intake rate decreased with the increase of tensile resistance of the stem component of the sward.

In swards with a stem-dominated lower stratum and a leaf-dominated upper stratum, the lower stratum can form a vertical barrier reducing bite depth (FLORES et al., 1993; BENVENUTTI et al., 2006, 2009).

It is easy to find in Brazilian conditions, environments where the criteria to start graze is lost, and the plant maturity increases. In that point, the NDF content increases and the nutritive value associated with more stem tensile resistance may contribute to decrease DMI.

The animal is able to choose the ideal point to graze, but some changes occur among the grazing process, regarding maximizes the forage intake and to do that, the animal is obligate to change for another point to graze. This point is called feeding station (FS), and is defined as the area of pasture a grazing animal can reach at each eating step. The eating step is taken while eating, considering that grazing activities involves eating and searching (SEARLE et al., 2005).

The bite profitability is defined as digestible energy harvested with each bite per handling time per bite (FORTIN, 2001). The decisions regarding the profitability of each bite influence departure decisions or residence time per FS (SEARLE et al., 2005). According to Wade and Gregorini (2010), this decline and the perception of FS more profitable in the surrounds motivate the animal to move to a new FS.

Therefore, a bite of forage should be more profitable to a hungry animal than to one that is not hungry (NEWMAN et al., 1994).

### **2.3 Effects of grazing management on animal performance**

There are a limited number of performance trials where the 95% light interception criterion was compared to fixed grazing interval with tropical grasses.

Gimenes et al. (2011) compared two sward heights as the start grazing point, 25 cm (95% light interception) and 35 cm (close to 100% light interception) for Nellore yearling bulls grazing *Brachiaria brizantha* cultivar Marandu (Palisade grass) (Table 3). In both treatments pastures were grazed down to 15 cm height.

Table 3 - Effect of start grazing point (25 vs 35 cm sward height) on performance of Nellore yearling bulls grazing Palisade grass (*Brachiaria Brizantha* cv. Marandu)

Sward height	ADG (kg/animal)	Stocking rate (450 kg AU/ha)	Kg LWG/ha
25 cm	0,957	2,88	621
35 cm	0,769	2,43	401

Average values for summer and fall season. Gimenes et al. (2011)

Animals grazing the pastures managed based on the 95% light interception criterion (25 cm as the grazing starting point) presented greater ADG and the pastures supported greater stocking rate. The fine tuning on pasture management resulted in 55% increase in BWG/ha.

Voltolini et al. (2010) reported higher milk production, higher stocking rates and consequently higher productivity (production per area) when cows grazed pastures of *Elephant grass* cv. Cameroon managed using 95% LI (103 cm sward height) as the grazing starting point in comparison with cows grazing paddocks managed with 27 days fixed grazing intervals (Table 4). Post grazing sward heights were 62 and 71 cm for the 95% LI and for the 27 d fixed GI respectively.

Table 4 - Effect of start grazing point (103 cm of sward height vs 27 days fixed grazing interval) on performance of dairy cows grazing Elephant grass cv. Camerron

	27 days of grazing interval	95% LI 1,03 m of sward height	P value
3.5% FCM, kg. cow d <sup>-1</sup>	14.88	17.65	0.1000
Cows.ha <sup>-1</sup>	5.1	7.2	0.0020
Milk, kg.ha.d <sup>-1</sup>	75	114	0.0004

Voltolini et al. (2010)

Carvalho et al. (2001), Da Silva and Carvalho (2005), Casagrande (2010), Vieira (2011) and de Paula et al. (2012) reported greater DMI of cattle grazing

pastures with better sward structure. Carvalho (1997) and Pedreira et al. (2005) reported that cattle grazing pastures with same initial forage mass but with different sward structures may have different forage intake and performance.

## 2.4 Effects of supplementation on forage intake and ruminal fermentation

Energy supplementation is often practiced during summer or wet season, where the forage has higher CP content compared with winter or dry season. In well managed tropical grass, with high doses of nitrogen fertilization and harvest at the ideal point (LI concepts) the CP content may be increased and in most cases the level of CP is enough to supply CP requirements for a growing beef cattle, being the energy the most limiting nutrient, since it is not possible to reduce substantially the NDF content, that vary from 54.2 to 66.3% (Table 5).

Table 5 - Chemical composition (% DM) of hand plucked or grazing horizon samples of well managed tropical pastures collected during spring, summer and fall

Forage	CP <sup>1</sup> , %	NDF <sup>2</sup> , %	Reference
<i>Brachiaria brizantha</i> cv. Marandu	12.6	57.4	Correia (2006)
<i>Brachiaria brizantha</i> cv. Marandu	13.6	56.2	Correia (2006)
<i>Brachiaria brizantha</i> cv. Marandu	15.3	65.0	Costa (2007)
<i>Brachiaria brizantha</i> cv. Marandu	15.4	63.9	Pacheco, jr. (2009)
<i>Brachiaria brizantha</i> cv. Marandu	11.9	66.3	Agostinho neto (2010)
<i>Brachiaria brizantha</i> cv. Marandu	13.1	62.6	Dórea (2011)
<i>Pennisetum purpureum</i> cv. Cameroon	13.7	62.9	Martinez (2004)
<i>Pennisetum purpureum</i> cv. Cameroon	14.6	65.1	Voltolini (2006)
<i>Pennisetum purpureum</i> cv. Cameroon	20.6	63.2	Carareto (2007)
<i>Pennisetum purpureum</i> cv. Cameroon	17.6	64.4	Romero (2008)
<i>Pennisetum purpureum</i> cv. Cameroon	18.5	61.4	Martinez (2008)
<i>Pennisetum purpureum</i> cv. Cameroon	18.5	58.7	Danés (2010)
<i>Pennisetum purpureum</i> cv. Cameroon	15.5	60.2	Chagas (2011)
<i>Pennisetum purpureum</i> cv. Cameroon	18.6	54.4	Macedo (2012)
<i>Pennisetum purpureum</i> cv. Cameroon	18.3	54.2	Souza (2014)

<sup>1</sup>CP = Crude protein; <sup>2</sup>NDF = Neutral Detergent Fiber

According to Danés et al. (2013) and NRC (1996) cattle grazing only tropical grass normally are under imbalance between protein and fermentable carbohydrate. In well managed tropical grass, as mentioned above (Table 6), this fact it is intensified, because the CP content increases, but the NDF does not decrease in order to provide improvements in microbial synthesis as resulted of more available energy into the rumen (SANTOS et al., 2014).

Supply additional energy through concentrate supplement is a powerful tool to improve energy intake, minimizing the mentioned unbalance between protein and energy. However, providing additional energy in the form of supplement has often produced reductions in intake of grazed forage. Chase and Hibberd (1987) fed incremental levels of corn to cows consuming low-quality forage and reported linear decreases in forage OM intake. These results support observations from earlier work on energy supplementation (LUSBY; WAGNER, 1986). After that, Pordomingo et al. (1991) reported that cattle supplemented with corn while grazing summer pasture had reduced forage intakes. These reports above agree with other data from tropical and temperate forages (MINSON, 1990).

Matejovsky and Sanson (1995) and Henning et al. (1990) reported that low levels of energy supplementation for sheep increased intake. In general, as level of energy supplement provided increases, intake usually decreases. According to Caton and Dhuyvetter (1997) low levels of energy supplementation increase forage intake seem to occur much more frequently in studies with sheep than in those with cattle. Reductions in forage intake associated with corn supplementation have been attributed to starch (CATON; DHUYVETTER, 1997). The increases on levels of corn starch decreased forage intake in steers (SANSON et al., 1990). These reductions have been attributed to either depressions in ruminal pH or a carbohydrate effect (MOULD et al., 1983). Declining ruminal pH associated with increasing dietary starch should affect the ruminal bacteria toward greater amylolytic and lower cellulolytic population. Resulting bacterial shifts are thought to reduce fiber digestion and negatively affect intake of grazed forage.

These hypotheses initially mentioned by many nutritionists that energy supplementation reduces rumen pH, affecting fiber digestion and consequently forage DMI is not consistent according to reviews published by Caton and Dhuyvetter (1997) and Santos et al. (2009). Studies regarding the factors that regulates

voluntary intake have been focus by many researchers during the last years (ANIL; FORBES, 1988; ALLEN et al., 2009; ZIEBA et al., 2005).

Energy supplementation is responsible for increasing volatile fatty acids, such as propionate, what is pointed out as one of the responsible to modulate dry matter intake in ruminants. The hypophagic effect of propionate in ruminants has been widely documented (ALLEN et al., 2009). However, information regarding the exact mechanisms of propionate in the liver and its action on the hungry control is scarce.

The voluntary intake can be regulated by hormones that affect the satiety status, such as insulin, leptin and ghrelin, secreted by pancreas, adipose tissues and stomach (abomasum in ruminants). These hormones are sensible to diet manipulation, and its believe that they play an important role in front of to send signals for central nervous system regarding the energy status and then regulates the DMI (ZIEBA et al., 2005).

For example, Ghrelin has recently been suggested as a powerful, peripherally active, orexigenic agent (WREN et al., 2000; KOBELT et al., 2006; ROCHE et al., 2007). Plasma ghrelin concentration increases in response to fasting and decreases during subsequent feeding in humans (CUMMINGS et al., 2001) and ruminants (HAYASHIDA et al., 2001; WERTZ-LUTZ et al., 2006; ROCHE et al., 2007). The orexigenic property of ghrelin may be involved in determining feeding mechanisms and behavior (NAKAZATO et al., 2001; SUGINO et al., 2004) as well as energy balance (HOSADA et al., 2002), the latter being related to glucose and insulin metabolism (MENDEZ et al., 2006; TAKAHASHI et al., 2006). The positive relationship between plasma ghrelin concentrations and DMI has been demonstrated in confined sheep (MENDEZ et al., 2006).

Gregorini et al. (2009) conducted a study aiming to investigate changes in foraging behavior, hunger-related hormones, and metabolites of dairy cows in response to short-term variations in rumen fill. These authors found that when RF was decreased, the glucose levels declined and ghrelin increased. Insulin acts as both a short- and long-term regulator of feed intake (WOODS et al., 1979). The results of Gregorini et al (2009) study add to this effect, directly or indirectly relating the physical stimuli coming from the rumen to the role of this hormone in intake regulation.

Gregorini et al. (2009) found correlation between insulin concentration and bite depth and tended to be associated with bite mass. This fact demonstrates that many

alterations on DMI in grazing animals are not caused just by rumen fermentation, but by hormonal regulation that can affect the grazing behavior and then de DMI. Thus, for many years the rumen fermentation was the focus of possible explanations to reductions on dry matter intake in supplemented animals, even in normal conditions (rumen pH, ammonia, level of CP, etc.). The Dr. Gregorini's group has indicated that cattle respond to an energy acquisition stimulus by changing ingestive behavior, what many times is resulted of interactions between energy status and grazing management, which has the hormones as an important regulators.

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### 3 FEEDING LOW LEVEL OF ENERGY SUPPLEMENT FOR BEEF CATTLE AND INTERACTIONS WITH GRAZING MANAGEMENT

#### Abstract

Eight 24-month-old rumen-cannulated Nellore steers (300 kg BW  $\pm$  5.97) were used in two 4x4 Latin squares to evaluate the interaction between energy supplementation and pre-grazing sward height on cattle grazing behavior, nutrient intake, digestion and metabolism. Treatments corresponded to 0 (mineral supplementation) or 0.3% (0.3% of BW of ground corn on a DM basis) combined with 2 pre-grazing sward heights (25 and 35 cm), with a common stubble height of 15 cm for all treatments. Steers were managed on 2 ha of Palisadegrass pasture (*Brachiaria brizantha* cv. Marandu) from February to April of 2011. Pastures were fertilized with 120 kg of nitrogen per ha and averaged 13.8 and 11.0% CP and 58.8 and 63.4% NDF, for the 25 and 35 cm grazing treatments, respectively. The parameters evaluated were forage intake, rumen pH and N-NH<sub>3</sub>, microbial synthesis, VFA, blood glucose, nitrogen balance and grazing behavior. Forage DMI and apparent DM, CP and NDF digestibility were obtained using Cr<sub>2</sub>O<sub>5</sub> and indigestible NDF as digesta markers. Concentration of purine derivative in the urine was used to estimate microbial synthesis. Forage DMI was increased (1.86 vs 1.32 P<0.05) when pre-grazing sward height was 25 cm and decreased when 0.3% BW supplement was fed (1.79 vs 1.38, P<0.05). The total and digestible DMI were not affected by energy supplementation (P>0.05), however they were increased (P<0.05) when pre-grazing sward height was 25 cm. Steers grazing the 25 cm treatment spent significantly less time grazing and more time resting (P<0.05), took less steps between feeding stations and per day (P<0.05) and presented greater bite rates (P<0.05) when compared with 35 cm treatments. Energy supplementation decreased grazing time (P<0.05), but it did not affect any other grazing behavior parameter. The DM and CP digestibility of forage and of total diet were greater (P<0.05) for the 25 than the 35 cm grazing management. Energy supplementation increased (P<0.05) diet DM digestibility, without effects on CP and NDF digestibility (P>0.05). Rumen pH (6.39 vs 6.52) was less (P<0.05) and rumen N-NH<sub>3</sub> (11.22 vs 9.77 mg/dL) was greater (P<0.05) for the 25 cm grazing management. Supplementation decreased (P<0.05) rumen N-NH<sub>3</sub>. Microbial synthesis was not affected (P>0.05) by treatments. The nitrogen retention was increased (P<0.05) for the 25 cm grazing management and not affected by supplementation (P>0.05). Concentration of total VFA was not affected by treatments (P>0.05). However, the energy supplementation decreased rumen acetate and increased propionate (mol/100 mol), resulting in decreased A:P ratio (P<0.05). Blood glucose was not affected by treatments (P>0.05). The pre-grazing sward height of 25 cm was effective to improve cattle harvesting efficiency and energy intake, but there was no benefit of feeding low level of energy supplement on energy intake of cattle on tropical pasture under rotational grazing.

Keywords: Beef cattle; Energy; Grazing; Management; Supplementation; Tropical grass

### 3.1 Introduction

Forage intake is regulated by physical effect or rumen fill, or physiological effect. For grazing cattle, where the most part of the diet is composed by fiber, the physical regulation is the major mechanism controlling forage intake (CONRAD et al., 1964).

However, for grazing cattle, before the forage can reach the gastrointestinal tract, it has to be harvested by the animal (CANGIANO et al., 2002). The data compilation done by Hodgson et al. (1977) suggested that the structure of the sward may have a greater impact on forage intake of grazing cattle than the physiological mechanisms discussed by Conrad et al. (1964) and Allen (2000).

The structure of the sward has a great impact on the efficiency of forage harvesting by grazing cattle, either for temperate grasses (HODGSON et al., 1994) as for tropical grasses (DA SILVA; PEDREIRA, 1996; DA SILVA; CARVALHO et al., 2001; CARVALHO, 2005; DA SILVA et al., 2009; PAULA et al., 2012). An important point reported by Pedreira et al. (2005) is that cattle grazing pastures with same initial forage mass but with different sward structures may have different forage intake and performance.

The concept of an ideal start grazing point originated from studies with temperate grasses (HODGSON, 1990) using 95% light interception (LI) criterion has been successfully applied to tropical grasses (DA SILVA; NASCIMENTO JÚNIOR, 2007). The latter authors reported a number of studies conducted in Brazil with various tropical grasses and a range of sward height values correlated to 95% LI (CARNEVALLI et al., 2006; BARBOSA et al., 2007; TRINDADE, 2007). According to (DA SILVA; NASCIMENTO JÚNIOR, 2007) pastures managed based on the 95% LI criterion present a sward that allows cattle to harvest greater amount of forage in a shorter grazing period.

Even in intensive tropical grazing systems based on well managed pastures as discussed above, animal performance is still far lower than animal's genetic potential. Most of this limitation comes from limited energy intake (DANÉS et al., 2013), due to limitations in forage harvest capacity of the animals and rumen fill caused by fiber content of the tropical grasses (WILSON, 1994). Feeding concentrates high in energy is a powerful tool to increase energy intake, stocking rate and cattle performance (VENDRAMINI et al., 2006, 2007; DA CRUZ et al., 2009;

FERNANDES et al., 2010). However interactions between energy supplementation and grazing management have not been studied for tropical grasses. Therefore, the objectives of this study were to evaluate the effects of feeding an energy supplement at 0 or 0.3% BW and its interaction with grazing management on cattle: 1) ingestive behavior; 2) nutrient intake; 3) rumen parameters; 4) blood glucose; 5) apparent digestibility; 6) rumen fermentation kinetics; and 7) nitrogen use.

### **3.2 Materials and methods**

This article is the first of a set of 2 experiments that evaluated interactions between energy supplementation and grazing management. This article used a low level of energy supplementation (0.3% BW), while in the companion article a higher level of energy supplementation (0.6% BW) was tested. The experiments had independent animals, but were carried out simultaneously in the same grazing area. Thus, the forage chemical and morphological composition were the same for both and will be presented in the current paper.

#### **3.2.1 Experimental area and animals**

The experiment was conducted at the Department of Animal Science of the University of São Paulo (USP), located in Piracicaba-SP, Brazil, during the months of February to April of 2011. The experimental area consisted of 2 ha of Palisadegrass cv marandu managed as a rotational grazing system divided in 16 paddocks and 2 treatments (25 and 35 cm pre-grazing sward height). Eight 24-month-old Nellore steers, 300 kg BW  $\pm$  5.97, cannulated in the rumen were used in this study.

#### **3.2.2 Experimental treatments and management practices**

Treatments consisted of 2 levels of energy supplementation (mineral supplementation or 0.3% BW of ground corn as fed basis combined with 2 pre-grazing sward heights (25 and 35 cm), with a common stubble height of 15 cm for all treatments. Pasture management was performed as a rotational grazing system, with use of nitrogen fertilizer (40 kg/ha per grazing cycle). The targeted pre-grazing sward height of 25 and 35 cm was based on research with Palisadegrass cv. Marandu correlating these measurements to 95 and 100% light interception (TRINDADE, 2007).

The experiment lasted 64 d and consisted of four experimental periods of 16 d each. Animals were weighed (full BW) prior to the start of each experimental period and the allocation of supplement was fixed for that experimental period. The first 8 d were used to adapt the animals to the experimental treatments. The following 8 d consisted of sample collections in the following order: on days 9 to 13 the fecal samples collection was performed, on day 14 measurements of animal behavior were taken, on day 15 collections of blood and urine samples were performed and on day 16 rumen fluid samples were collected. Animals had free access to water and minerals supplement in every paddock. The supplemented and the no supplemented experimental animals were brought daily to a management center close to the experimental area where the supplement was offered in individual feed bunks from 1000h to 1040h. The no supplemented steers were also contained in the feed bunks. This management center was also utilized for infusions of external marker and samplings.

### **3.2.3 Feed Samples Collection, Chemical Analysis and Calculations**

Forage mass and its morphological composition were determined in every grazing cycle, before the steers entered every paddock and after they left. In each evaluation, 3 randomized points were selected in the paddock, and in each point, the material within a rectangle frame (0.5 m<sup>2</sup>) was cut to ground level. Total forage mass was weighted, 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. Pre and post-grazing sward height, forage mass, and morphological composition are presented in Table 6.

Table 6 - Forage mass, sward height and morphological composition of pre and post-grazing condition

	Pre-grazing sward height		SEM
	25 cm	35 cm	
Pre-grazing conditions			
Forage mass, kg DM/ha	8036.09	8899.75	530.36
Leaves, kg DM/há	3567.22	3306.59	194.67
Stems, kg DM/há	1405.51	1609.00	114.48
Dead material, kg DM/ha	3063.36	3984.16	457.21
Leaves, %	44.39	37.88	2.60
Stems, %	17.49	18.20	1.05
Dead material, %	38.12	43.91	2.96
Post-grazing conditions			
Forage mass, kg DM/ha	4331.02	4704.38	529.99
Leaves, kg DM/há	990.23	787.37	148.55
Stems, kg DM/há	782.78	1046.43	151.42
Dead material, kg DM/ha	2558.01	2870.53	373.39
Leaves, %	22.89	16.85	2.30
Stems, %	18.09	22.39	2.37
Dead material, %	59.12	61.42	3.63
Sward height			
Pre-grazing, cm	26.01	37.21	1.63
Post-grazing, cm	14.61	17.45	0.93

<sup>1</sup>Standard error of the means

The forage mass on pre-grazing conditions for the 25 cm treatment was around 900 kg/ha greater than values reported in the literature for the same grass, grazing management and season (SOUZA JÚNIOR, 2007; TRINDADE, 2007). The mass on the pre and post-grazing conditions can vary depending on the adopted management before the start of the trial, such as level of nitrogen fertilization, season, stocking rate, stubble height, and other factors.

For pastures managed at 25 cm of pre-grazing sward height, the leaves disappearance was 2,576.99 kg DM/ha, while at 35 cm it was 2,519.22 kg/ha. Despite the similar amounts of leaves disappearing from both pasture treatments,

forage DMI may be less and forage loss may be greater for the 35 cm, considering that more stems may contribute as horizontal barrier, decreasing bite area, bite rate and consequently forage intake (DRESCHER et al., 2006; BAGGIO et al., 2009; BENVENUTTI et al., 2009).

For chemical composition analysis pasture samples (grazing horizon 15 to 25 and 15 to 35 cm) were cut and collected using a rectangle frame (0.5 m<sup>2</sup>), from 3 distinct points prior to animals' entry in every paddocks for every grazing cycle. Every batch of ground corn was sampled. Feed samples were oven dried at 55°C for 72 hours and ground through a 1 mm screen (Wiley mill). Pasture samples were bulked as one composite sample by treatment. Corn samples were bulked as one composite sample. Residual moisture content was determined by drying samples at 105°C for 24 h. Organic matter content of samples was determined after incineration at 550°C for 8 h in a muffle furnace (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC, 1990). Nitrogen content was determined by the Dumas combustion method (Leco 2000, Leco Instruments Inc) and a conversion factor of 6.25 was used to convert total nitrogen to CP. Contents of NDF, ADF and lignin were determined according to Van Soest et al. (1991) with no sulphite for all samples analyses and the addition of amylase for corn grain analyses only. The NDIN and ADIN were determined using micro Kjeldahl (AOAC, 1990) after digestion of samples with neutral (no sulphite) and acid detergents (VAN SOEST et al., 1991). The TDN values of feed samples were calculated according to Weiss et al. (1992). Forage samples were also analyzed for soluble nitrogen (KRISHNAMOORTY et al., 1982) and for non-protein nitrogen (NPN) (LICITRA et al., 1996) to estimate the protein fractions according to CNCPS (SNIFFEN et al, 1992).

#### **3.2.4 Rumen Fluid and Blood Samples**

Samples of rumen fluid were collected to determine N-NH<sub>3</sub> and VFA concentrations. On day 16 of every experimental period rumen fluid samples (approximately 100 mL) were collected at 0, 2, 4, 6, and 8 h after supplement feeding with the use of a sampling probe (DANÉS et al., 2013). The rumen pH was measured immediately and samples were frozen at -20°C for further analyses. Rumen fluid samples were thawed at room temperature centrifuged (15,000 g, 4°C, 30 min) and

analyzed for VFA using gas chromatography (PALMIQUIST; CONRAD, 1971), and for N-NH<sub>3</sub> by phenol-hypochlorite procedure (CHANEY; MARBACH, 1962).

Blood samples were collected on day 15 of the experimental period 4 h after feeding, from the coccygeal vessels 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. Levels of plasma glucose were analyzed by automatic biochemical analyzer YSI 2700 Select.

### **3.2.5 Forage Intake and digestibility**

Forage intake was calculated from total fecal excretion and the digestibility of feeds. On day 1 and for 13 consecutive days, chromium oxide (Cr<sub>2</sub>O<sub>3</sub>), previously weighed in two paper capsules (5 g each), was dosed into the rumen to all steers, one dose right after supplement feeding in the morning (1000 h) and a second dose in the afternoon (1700 h). Fecal grab samples (approximately 200 g per animal) were collected between days 9 to 13, twice a day (1000 h and 1700 h), dried at 55°C for 72 h and ground through a 1-mm screen (Wiley mill). Equal amounts of fecal DM 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 (CASALI et al., 2008). The CP and NDF concentrations were analyzed in the feces and multiplied by fecal production, to calculate CP and NDF digestibility.

For 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 RO water (VEGA; POPPI, 1997). The digested samples were analyzed using an inductively coupled plasma spectrometer (ICP-OES).

Forage intake was calculated dividing forage fecal excretion (total fecal excretion minus the amount resultant from the supplement) by the forage indigestibility.

### **3.2.6 Animal Behavior**

Grazing behavior measurements took place on d 14 of each period. Four trained observers were assigned to visually determine grazing (eating plus

searching), rumination, and idling time every 5 min for consecutive 24 hours. The number of observations for each activity during a 24 hours period was multiplied by 5 minutes to get the daily amount of time spent in that activity. While grazing, cattle search, acquire into the mouth, masticate, and swallow herbage (GIBB, 1998). The observers also counted bite rate (bites/min) using the time spent to 20 bites (PENNING; RUTTER, 2004).

Each eating step was considered a feeding station (RUYLE; DWYER, 1985; ROOK et al., 2004). The feeding stations per minute were calculated considering the time spent to 10 feeding station divided by 10. Also, it was measured the number of steps between feeding stations, and then it was calculated the total steps per day, multiplying the steps per minute by grazing time (min).

### **3.2.7 Microbial Protein Synthesis and Nitrogen Efficiency**

Nitrogen intake was calculated by the sum of intakes of N from forage and supplement origins. Apparent efficiency of N use (assuming no retention or mobilization of N in the body) was calculated for every steer by dividing mean retained N (N intake minus N in urine and feces) by the average N intake.

Microbial synthesis was estimated through the excretion of purine derivatives (STANGASSINGER et al., 1995). Spot urine samples were collected on day 15 of each experimental period, in a single sample collected approximately 4 h after supplement feeding. Sub-samples of urine (10mL) 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 a HPLC (PIMPA; BALCELLS, 2002), and for N using micro Kjeldhal (AOAC, 1990).

Total urine production was estimated using the creatinine content in the sample, assuming that excretion of creatinine is constant (0.213 mmol kg BW<sup>-1</sup>) (CHIZZOTTI et al, 2008). The excretion of purine derivatives (PD) was then calculated as the sum of allantoin and uric acid excreted in urine, expressed in mmol/d. The analyses of PD were performed according to Pimpa et al. (2001) using values of 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).

### 3.2.8 Rumen Fermentation Kinetics

The *in vitro* degradation rate of pasture sample was estimated using the semi-automated method of cumulative gas production adapted by Mauricio et al. (1999). Four replicates of approximately 1 g of feed samples of 2 pre-grazing sward height (25 and 35 cm) were incubated using 10 ml of rumen inoculum obtained from a steer grazing Marandu palisadegrass and fed 1 kg/d of supplement containing ground corn, soybean meal, urea and minerals, mixed with 90 ml of batch culture.

The pressure of produced gases was measured at 1, 2, 3, 4, 6, 8, 10, 12, 14, 17, 20, 24, 28, 36, 48, 72, 96 and 120 hours after incubation using a pressure gauge transducer (PDL800). The value obtained from each reading was then subtracted from a standard value (sample with no substrate). The bicompartamental logistic model proposed by Pell and Schofield (1993) was used to predict the final volume of gas from the degradation of fibrous carbohydrates (VFC), the total gas production from the degradation of non-fibrous carbohydrates (VNFC), lag time (L), and the degradation rate of fibrous and non-fibrous carbohydrates (KdFC and KdNFC, respectively).

### 3.2.9 Statistical Analysis

The experimental design was a double 4x4 Latin Square design, with eight animals, four treatments and four periods. All variables, except the rumen fermentation kinetics and its predicted parameters, were analyzed using the PROC MIXED package of SAS statistical system (SAS<sup>®</sup> version 9.2<sup>®</sup>, 2008). The rumen fermentation kinetics variables were analyzed in a completely randomized design using PROC GLM package of SAS statistical system (SAS<sup>®</sup> version 9.2<sup>®</sup>, 2008). The model included treatment, animal and period as sources of variation to analyze the data of forage, nutrient intake, animal behavior, N excretion, blood parameters and microbial synthesis. Treatments and periods were considered fixed, while animal and overall errors were considered random effects. The model used to analyze rumen parameters (pH and concentrations of VFA and NH<sub>3</sub>-N) collected at different times after feeding, included treatment, period, animal, hours post-feeding as repeated measures and treatment x hours post-feeding interaction as sources of variation.

All variables were considered fixed, except animal, whole-plot error, and subplot error, which were considered random. The covariance structure used for

repeated measures analyses was chosen based on Bayesian Information Criteria parameter of SAS statistical system (SAS<sup>®</sup> version 9.2<sup>®</sup>, 2008) closer to zero.

The averages were calculated by Least squares means and adjusted for comparison by Tukey test at 5% and 10% probability. Thus, significance was declared at  $P < 0.05$  and  $P < 0.01$ .

The parameters of rumen fermentation kinetics predicted by the equations were fitted by nonlinear models using PROC NLIN of the SAS statistical system (SAS<sup>®</sup> version 9.2<sup>®</sup>, 2008). Necessarily, all data sets were tested before the final overall analysis to ensure that all the assumptions of analysis of variance (additive model, independence of errors, data normality and homoscedasticity) were met.

### 3.3 Results

#### 3.3.1 Pasture and Supplement Samples

The forage CP, NDF and TDN values for 25 cm grazing management were 13.8, 58.8 and 57.3%, and for 35 cm were 11.0, 63.4 and 54.9%, respectively (Table 7). Even though the CP content for 35 cm had been slightly lower than for 25 cm, the A, B and C fractions were similar between 25 and 35 pre-grazing sward height (Fraction A: 27.5 and 26.7 % CP, Fraction B: 63.5 and 64.0, Fraction C: 8.9 and 9.2, respectively) (Table 8). The energy supplement contained 9.3% CP and 87.3% TDN.

Table 7 - Composition of forage (grazing horizon samples) and ground corn

	Pre-grazing sward height		Ground corn
	25 cm	35 cm	
Ash, % DM	9.97	9.34	0.90
CP, % DM	13.87	11.02	9.35
EE, % DM	1.18	0.93	3.67
NDF, % DM	58.80	63.40	11.17
ADF, % DM	33.05	35.85	2.93
HEM, % DM	25.76	27.55	8.24
CEL, % DM	29.96	32.44	2.39
Lignin, % DM	3.29	3.40	0.54
TDN, % DM	57.33	54.89	87.34

<sup>†</sup>Total digestible nutrients (WEISS et al., 1992)

Table 8 - Carbohydrates and protein fractions of forage (grazing horizon samples)

Protein Fractions	Pre-grazing sward height, cm		Carbohydrates Fractions	Pre-grazing sward height, cm	
	25	35		25	35
	A, % CP	27.57		26.72	CHOT <sup>1</sup> , % DM
B1, % CP	12.65	7.59	A+B1, % CHOT	10.73	6.91
B2, % CP	37.84	49.61	B2, % CHOT	78.39	79.79
B3, % CP	13.02	6.85	C, % CHOT	10.88	13.29
C, % CP	8.93	9.24			

### 3.3.2 Grazing Behavior and Feed Intake

There was no interaction between grazing management and level of energy supplementation ( $P>0.05$ ) for grazing behavior (Table 9) and feed intake (Table 10). Animals grazing pastures managed with 25 cm sward height spent less time on grazing activity and spent more time resting and had greater bite rates ( $P<0.05$ ) compared to cattle grazing pasture with 35 cm sward height as the start grazing point (Table 9).

The number of feeding stations per minute was greater ( $P<0.05$ ) for animals grazing pastures with 25 cm, but with less steps between feeding stations. The steps per minutes were not affected by grazing management ( $P>0.05$ ). However, as consequence of longer grazing time for the 35 cm treatment the total steps per day also was greater for 35 cm pre-grazing sward height, given the fact that steps/min did not change ( $P>0.05$ ).

Energy supplementation decreased ( $P<0.05$ ) grazing time but had no effects on the other grazing behavior variables ( $P>0.05$ ).

Table 9 - Effects of levels of energy supplementation and grazing management on grazing behavior

	Management, Cm		Level of supplementation, % BW		P value			SEM <sup>1</sup>
	25	35	0	0.3	M	S	M*S	
Grazing time	386.88	465.25	445.94	406.18	0.0001	0.0236	0.6380	20.29
Ruminating time	385.00	384.49	379.99	389.50	0.9767	0.5862	0.4167	19.73
Rest time	608.12	530.73	554.20	584.66	0.0005	0.1199	0.7240	18.33
Bite rate, bit/min	34.23	22.78	28.20	28.80	0.0003	0.8253	0.2945	3.28
Feeding stations/min	5.06	4.13	4.61	4.58	0.0145	0.9296	0.9849	0.35
Steps between feeding stations	1.29	1.60	1.52	1.38	0.0157	0.2457	0.2275	0.11
Steps/min	6.51	6.58	6.90	6.18	0.9201	0.2902	0.3446	0.49
Steps/day	2524.0	3009.8	2922.7	2611.0	0.0973	0.2803	0.6364	215.8

<sup>1</sup>Standard error of the means, M=management, S=supplementation, M\*S=interactions between management and supplementation

Cattle grazing pastures managed with 25 cm (correlated to 95% LI) had greater intakes ( $P<0.05$ ) of forage DM, total DM, digestible DM, NDF and CP than animals grazing pastures managed with 35 cm as a start grazing point (Table 10).

Supplementing cattle with ground corn at 0.3% BW reduced forage DMI ( $P<0.05$ ) and NDF intake ( $P<0.10$ ), with no effect ( $P>0.05$ ) on intakes of total DM, digestible DM, and CP. Feeding supplement at 0.3% BW resulted in a substitution rate of 1.63.

Table 10 - Effects of levels of energy supplementation and grazing management on intakes of forage DM, total DM, digestible DM, NDF and CP and on substitution rate

	Management, cm		Level of supplementation, % BW		P value			SEM <sup>1</sup>
	25	35	0	0.3	M	S	M*S	
Forage DMI, % BW	1.86	1.32	1.79	1.38	0.0104	0.0454	0.9905	0.14
Total DMI, % BW	2.01	1.47	1.79	1.68	0.0101	0.5788	0.9905	0.14
Digestible DMI, % BW	1.41	1.00	1.20	1.21	0.0094	0.9823	0.9347	0.08
NDF DMI, % BW	1.11	0.85	1.09	0.88	0.0381	0.0872	0.918	0.09
CP DMI, % BW	0.27	0.15	0.22	0.20	0.0001	0.3443	0.8105	0.01
Substitution rate <sup>2</sup>	0.00	0.00	0.00	1.63	0.9652	0.0019	0.8107	0.25
Forage DMI, kg/d	6.34	4.56	6.19	4.72	0.0015	0.0295	0.8307	0.53
Total DMI, kg/d	6.85	5.08	6.19	5.75	0.0096	0.4887	0.8241	0.54
Digestible DMI, kg/d	4.81	3.49	4.16	4.14	0.0041	0.9519	0.8985	0.35
NDF DMI, kg/d	3.79	2.94	3.76	2.97	0.0414	0.0542	0.7642	0.32
CP DMI, kg/d	0.92	0.55	0.78	0.69	0.0001	0.2857	0.9659	0.07

<sup>1</sup>Standard error of the means, <sup>2</sup>= kg of reduction on forage DMI for kg of supplemented fed, M=management, S=supplementation, M\*S=interactions between management and supplementation

### 3.3.3 Apparent digestibility

The forage and diet DM and CP digestibility were greater ( $P < 0.05$ ) for 25 cm pre-grazing sward height than for 35 cm (Table 11). Energy supplementation increased ( $P < 0.05$ ) diet DM digestibility ( $P < 0.05$ ), with no negative effect ( $P > 0.05$ ) on NDF digestibility.

Table 11 - Effects of energy supplementation and grazing management on forage and diet total tract digestibility

	Management, cm		Level of supplementation, % BW		P value			SEM <sup>1</sup>
	25	35	0	0.3	M	S	M*S	
Forage digestibility (%)								
DM	68.13	65.44	67.31	66.26	0.0013	0.1683	0.6518	0.78
CP	74.68	63.87	70.17	68.37	0.0001	0.1846	0.8509	0.94
NDF	63.42	62.57	62.44	63.55	0.5189	0.3946	0.5556	1.19
Diet digestibility (%)								
DM	70.13	68.43	67.31	71.25	0.0523	0.0001	0.1213	0.97
CP	75.85	66.55	70.20	72.21	0.0001	0.1391	0.1764	1.36
NDF	63.12	62.13	62.44	62.80	0.4458	0.7786	0.6332	1.16

<sup>1</sup>Standard error of the means, M=management, S=supplementation, M\*S=interactions between management and supplementation

### 3.3.4 Rumen Fluid and Plasma Glucose

There was no interaction between grazing management and energy supplementation ( $P>0.05$ ) for both rumen parameters and plasma glucose (Table 12).

The rumen pH was not affected by energy supplementation ( $P>0.05$ ), but it was decreased by the 25 cm grazing management ( $P<0.05$ ). The rumen N-NH<sub>3</sub> was greater ( $P<0.05$ ) for cattle grazing the pasture managed with 25 cm height as a start grazing point compared with the 35 cm height. Feeding ground corn to grazing cattle decreased ( $P<0.05$ ) rumen N-NH<sub>3</sub>. Total VFA concentration was not affected ( $P>0.05$ ) by treatments but supplementing energy decreased ( $P<0.05$ ) rumen acetate, increased ( $P<0.05$ ) rumen propionate and decreased ( $P<0.05$ ) acetate:propionate ratio. Plasma glucose was not affected by increasing energy supplementation and grazing management ( $P>0.05$ ).

Table 12 - Effects of energy supplementation and grazing management on rumen pH, N-NH<sub>3</sub>, volatile fatty acids (VFA) and plasma glucose

	Management, cm		Level of supplementation, % BW		<i>P value</i>			SEM <sup>1</sup>
	25	35	0	0.3	M	S	M*S	
pH	6.39	6.52	6.46	6.44	0.0015	0.5734	0.3432	0.14
NH <sub>3</sub> /N, mg/dL	11.22	9.77	11.28	9.70	0.0210	0.0120	0.3397	1.10
Total VFA,mM	145.09	145.55	144.40	146.04	0.3203	0.8211	0.7757	7.05
	VFA mol/100 mol							
Acetate	69.84	70.54	71.09	69.37	0.7026	0.0063	0.9879	0.90
Propionate	19.35	19.23	18.49	20.24	0.9516	0.0001	0.5198	0.53
Butyrate	10.98	10.27	10.42	10.70	0.2281	0.5715	0.9620	0.63
A:P	3.75	3.74	3.95	3.51	0.7488	0.0001	0.4043	0.13
Plasma glucose	51.77	53.21	54.30	53.89	0.9821	0.9590	0.6532	1.89

<sup>1</sup>Standard error of the means, M=management, S=supplementation, M\*S=interactions between management and supplementation

### 3.3.5 Microbial Protein Synthesis and Nitrogen Use

There was no interaction ( $P>0.05$ ) between energy supplementation and grazing management for nitrogen use and microbial synthesis (Table 13). Feeding only 0.3% BW of ground corn did not improve ( $P>0.05$ ) the use of N by cattle grazing fertilized Palisadegrass pasture. Microbial synthesis and efficiency ( $P>0.05$ ) (Table 13). However, managing the pasture with 25 cm pre-grazing sward height caused a huge increase ( $P<0.05$ ) in the intake of N with greater retention of N ( $P<0.05$ ) and greater efficiency of N utilization ( $P<0.05$ ) by cattle grazing fertilized Palisadegrass pasture.

Table 13 - Effects of levels of energy supplementation and grazing management on nitrogen intake, nitrogen urine, nitrogen feces, nitrogen retained (% N intake), microbial protein (MicP) and microbial efficiency (MicEf)

	Management, cm		Level of supplementation, % BW		<i>P value</i>			SEM <sup>1</sup>
	25	35	0	0.3	M	S	M*S	
N fecal <sup>2</sup>	35.34	34.86	36.9	33.29	0.9392	0.562	0.7578	8.8
N urinary <sup>2</sup>	41.99	44.62	44.73	41.89	0.6336	0.6336	0.7898	8.35
N excretion <sup>2</sup>	77.48	79.49	81.64	75.33	0.771	0.3653	0.6275	16.1
N intake <sup>2</sup>	155	100.41	130.2	125.21	0.0064	0.7855	0.892	24.11
N retention <sup>2</sup>	77.36	20.91	48.55	49.25	0.0089	0.9715	0.99	21.08
N retention, %N intake)	49.7	20.82	39.9	40.68	0.0446	0.9296	0.6626	6.66
MicP <sup>2</sup>	457.07	411.25	425.01	443.32	0.3114	0.6830	0.6163	62.35
MicEf. g/kg of TDN	111.97	145.63	130.29	127.30	0.0935	0.8776	0.4861	14.48

<sup>1</sup>Standard error of the means, <sup>2</sup>g/day, M=management, S=supplementation, M\*S=interactions between management and supplementation

### 3.3.6 Rumen Fermentation Kinetics

Forage samples from pastures managed with 25 cm height as a start grazing point presented shorter ( $P < 0.05$ ) lag time and greater ( $P < 0.05$ ) degradation rate of fibrous carbohydrate (Table 14) compared with forage from the 35 cm pastures.

Table 14 - Effect of pasture management on rumen kinetics fermentations parameters of Palisadegrass

Parameters *	Pre-grazing sward height		P value	SEM
	25 cm	35 cm		
fVFC, mL/g DM	121.4	119.3	0.5887	2.7
kdFC, %/h	0.0200	0.0182	0.0371	0.0008
fVNFC, mg/ g DM	107.3	100.8	0.3090	3.4
kdNFC, %/h	0.0718	0.0735	0.4842	0.0041
Lag time, h	5.4	6.0	0.0153	0.2

SEM= standard error of the means; fVCF= final volume of fibrous carbohydrate; kdCF= rate of degradation of fibrous carbohydrate; fVNFC= final volume of non-fibrous carbohydrate; kdNFC= rate of digestion of non-fibrous carbohydrate.\*Parameters estimated according to Pell and Schofield, 1993

### 3.4 Discussion

#### 3.4.1 Pasture and supplement samples

Many factors may affect the forage's nutritive value such as its physiological stage, the soil organic matter content and nitrogen applied as fertilizer (TAIZ; ZEIGER, 2004) altering especially the CP content (JONHSON et al., 2001; DANÉS et al., 2013). Research data, from studies using cattle grazing tropical grasses during the wet season reported NDF content varying from 58 up to 75% and CP content from 5 up to 22% (MORAIS et al., 2009; DANÉS et al., 2013; FLORES et al., 2008).

The forage contents of NDF and lignin were lower and the CP contents were higher than usually found in tropical grasses (TEDESCHI et al., 2002; MORAIS et al., 2009; VIEIRA et al., 2000; NASCIMENTO et al., 2009) as a consequence of intensive grazing management, N fertilization, and the sampling method (i.e., the part of the plant that was sampled).

When the pastures are managed to be grazed at their optimal point (meaning variable grazing intervals), compared with fixed intervals, which for tropical grasses are traditionally longer than the average of the variable ones, not only is the forage offered to the animal of better quality, but the sward structure also increases the grazing efficiency (CARNEVALLI et al., 2006). In this present study the CP, NDF, Lignin and TDN values for 25 cm pre-grazing sward height were 13.8, 58.8, 3.3 and 57.3%, respectively. For 35 cm of sward height these nutrients values were 11.0,

63.4, 3.4 and 54.8, respectively (Table 7). Both 25 and 35 cm pastures may be considered with medium nutritive value according to NRC (1996, 2001) standard values for high quality forage.

Recently, Lopes (2011) characterized the nutrient profile and fiber digestibility of the main tropical grasses produced under intensive rotational grazing management in Brazil. One-hundred six samples of Palisade grass (*Brachiaria brizantha*); Mulato grass (*Brachiaria hibrida*), Bermuda grass (*Cynodon dactylon*); African Bermuda grass (*Cynodon nlemfuensis*), Guinea grass (*Panicum maximum*) and Elephant grass (*Penisetum purpureum*) were evaluated. Tropical grasses presented 14 to 21% CP and 60 to 63 % NDF, averaged by grass species. Comparison between mean of in vitro NDF digestibility estimates of tropical grasses by time-point and alfalfa silage standard indicates that tropical grasses had greater fiber digestibility than the alfalfa silage. The author concluded that tropical forages subjected to intensive grazing management practices can be relatively high in crude protein content and in fiber digestibility.

Despite the lower IVNDFD, alfalfa presents lower NDF content with greater particle fragility. Allen (2000) and Kammes and Allen (2012) reported that lactating cows presented greater DMI with diets containing alfalfa silage compared to grass silage in spite of greater NDF digestibility for grass silage. The explanation was that the filling effect of alfalfa was less because of greater particle fragility, which decreased retention time in the rumen reticulum and resulted in less distention and greater DMI.

Cattles grazing only tropical grass are usually under imbalance between protein and fermentable carbohydrate (NRC, 1996; DANÉS et al., 2013). In well managed tropical grass this fact may be intensified, because the CP content increases, but the NDF does not decrease in order to provide improvements in microbial synthesis as resulted of more available energy into the rumen (SANTOS et al., 2014). Thus, in the current study is possible to observe that for the 25 cm grazing management the CP content was 13.8% with high A+B1 (40.22% of CP) fractions, which present fast degradation rates. However, the NDF content was 58.8%, with low A+B1 carbohydrate fractions (10.73% of CHO) what may suggest imbalance between protein and energy, and low efficiency on nutrient usage, mainly CP. The same situation happened for forage from the 35 cm pastures (Table 8).

According to NRC (1996), tropical grasses containing 12% CP or more, with 71% of that CP as rumen degradable protein (RDP), and up to 65% TDN, supply protein in excess (RDP and metabolizable protein) but limit the performance of yearling growing cattle because of energy shortage. The low content of carbohydrate A + B1 fraction associated with high levels of CP, high in RDP, in well managed marandu palisadegrass may result in considerable losses of protein in the form of N-NH<sub>3</sub> from the rumen (NRC, 1996). The higher is the level of N fertilization, the higher is the forage CP content (JONHSON et al., 1991), what may result in an increased imbalance between fermentable carbohydrate and RDP (RUSSELL et al., 1992). Supplementing rumen fermentable energy may be an effective tool to improve performance of grazing cattle and improve the utilization of forage protein.

### **3.4.2 Animal Behavior and Forage Intake**

The rumen fill effect, caused by high fiber content in grasses, has been pointed out as the main factor that limits the forage intake in grazing animals (STOBBS, 1973; POPPI et al., 1980, 1981a, 1981b, 1985). However, grazing animals live the daily challenge of harvesting their own feed, which is often limited by structural characteristics of the sward (HODGSON, 1977; ILLIUS; GORDON, 1987; CANGIANO et al., 2002). The sward structure starts limiting DMI even before the chemical composition of forage plays its role. Trindade (2007) evaluated the 95% and 100% LI as the start grazing point criteria for Palisadegrass (Marandu), which resulted in average sward height of approximately 25 cm and 35 cm, same as the used in this trial. The combination of 25 cm pre-grazing height with 15 cm post-grazing height resulted in greater proportions of leaves and less proportions of stem and dead material in the forage available to animals, compared to pastures managed with 100% LI (it would be 35 cm pre-grazing sward height) and 15 cm, respectively, what corroborate with the morphological composition presented in this current study, as showed in Table 6.

The increases on sward height from 25 to 35 cm decreased DMI (total, forage, digestible, NDF and CP, Table 10) as response of decreases on harvesting efficiency (Table 9). This reduction in efficiency during the grazing down process is the result of greater proportion of stems that may acts as a horizontal barrier causing reductions on bite rate and bite area (UNGAR et al., 2001; BENVENUTTI et al., 2006;

DRESCHER et al., 2006; BAGGIO et al., 2009; BENVENUTTI et al., 2009) as observed for the bite rate values at 25 cm (34.23 bites/min) and 35 cm (22.78 bites/min) grazing managements (Table 9). Baggio et al. (2009) also reported reductions in bite rates as sward height increased. According to Carvalho (1997) the forage mass is proportional inversely a bite rate, once that in pastures with highest sward height and bite mass, the animal spend more time manipulating and chewing. This fact results in more time spent for each bite interval, what reduce the bite rate.

In the mentioned study conducted by Trindade (2007) it was reported greater proportions of leaves throughout the grazing down process in the extrusa of animals from 25 cm treatment, while animals grazing paddocks managed using 100% LI (or 35 cm) as the pre-grazing starting point had a greater participation of stems and dead material in their extrusa. Trindade (2007) results are in agreement with founds in this current study.

Animals grazing pastures managed at 25 cm had 78 minutes less for grazing time and 77 minutes more for resting time (Table 9). The difficult caused by stems during the grazing down process leaded the animals to increase the grazing time, such as happened for 35 cm treatments. However, even with possible compensations in bite mass, animals grazing pastures managed at 35 cm of sward height were not able to increase their DMI in comparison with 25 cm treatment.

The combinations between high bite rates and low grazing time suggest greater harvesting efficiency, what may be confirmed with greater DMI (Table 10) for animals grazing pastures with 25 cm of sward height. Also, this information suggest less energy expenditure for grazing activities for 25 cm treatments, supported by all mentioned parameters, mainly by greater resting time. Gimenes et al. (2011) evaluated the animal performance of Nelore bulls grazing Palisadegrass managed at the same pre-grazing sward height (25 vs 35 cm) during one year and they found the ADG 120 g more for animals that grazed pastures managed at 25 cm of sward height. Also, they found differences in stocking rate between 25 and 35 cm (3.13 and 2.85 AU/ha, respectively) and live weight production (886 and 674 kg/ha, respectively). The authors attributed the greatest response with 25 cm of sward height for greater harvesting efficiency, pointing the importance of grazing management on DMI and animal performance.

Two previous studies conducted at ESALQ/USP facilities found higher milk yields when cows were managed under height-based variables than when they were

managed under fixed grazing intervals (CARARETO, 2007; VOLTOLINI et al., 2010). The improvements on milk yield are attributed to greatest harvesting efficiency, once that the forage nutritive value were similar between grazing managements. In addition, the pre-grazing sward height average was higher for fixed grazing intervals management than for height-based (or LI concepts).

Animals grazing pastures managed at 35 cm spent more time per feeding station, resulting in less feeding station per min (Table 9), what can be related with more forage mass and the time spent to manipulating it in each feeding station (BAGGIO et al., 2009). The number of steps between feeding station and the total steps per day found for 35 cm treatments showed that animals walked more searching for better feeding stations. 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 feed station determines the time spent by the animal at the feed station (BAGGIO et al., 2009). Others authors also characterize this feeding station usage as feeding station profitability (WADE; GREGORINI, 2010, GREGORINI et al., 2011). Thus, animals start grazing at one feed stations, keeping it there until the forage allowance starts to get reduced, after that animals give up to keeping harvest and choose another feeding station (SEARLE et al., 2005). The distance walked between feeding stations may indicate how difficult is to find another new feeding station.

There was no effect of energy supplementation on feeding station/min, steps between feeding stations, steps/min and steps/day. In contrast, some authors found effects of supplementation on displacement patterns, where non-supplemented beef heifers traveled greater distances and visited more feeding stations per minute, while the opposite happened for supplemented animals (GLIENKE et al., 2010). A hypothesis raised was that animals spent less time searching for feeding stations because they had their nutritional requirements met. In the other hand, other study reported that supplemented animals were more selective and covered greater distances in search for better feeding stations (ADAMS, 1985).

The energy supplementation fed at 0.3% BW affected forage DMI for both 25 and 35 cm grazing managements, without interactions (grazing management\*supplementation) (Table 10) Thus, the energy supplementation decreased just the forage DMI as consequence of substitution effect, without increments in total and digestible DMI.

The intake regulation mechanism may be altered when supplements are used, because at certain levels of supplementation, the caloric density of the diet is expected to increase and control DMI through physiological effect mechanisms (MOORE et al., 1999). The physiological control mechanism apparently did not take effect at the used levels of supplementation (0.3% BW) in this study, since total DMI was not decreased. However, the lack of increases on digestible DMI may result in negative effects because one of the most important objectives to use energy supplementation is to increase the energy DMI and then animal performance.

Some theories were discussed in the past regarding the use of supplements to lead decreases on rumen pH, resulting in DMI reduction (ULMER et al., 1990; LEVENTINI et al., 1990). These observations does not corroborate with the results of this study, since corn supplementation caused no significant reductions in rumen pH, and no negative effects on fiber degradation (Table 11 and Table 12) but in spite of that, there had been decreases on DMI. Thus, it's in accordance with the literature that shows no reduction on ruminal pH below 6.2, but decreases on forage DMI (CHASE; HIBBERD, 1987; PORDOMINGO et al., 1991; ELIZALDE et al., 1998). However, other factors, such as hormonal regulation and propionate concentration (ALLEN et al., 2009), besides pH, acting on DMI reduction may play a more important role.

One important point in this study is related with the grazing method (rotational grazing system) used, that could have increased the substitution rates during the end grazing period, promoted by reductions on forage allowance along abasement. In studies conducted using another grazing method (continuous grazing system) the response on substitution rate when 0.3% BW was fed was different (CASAGRANDE, 2010; VIEIRA, 2011), suggesting that animals suffer a great effect of low levels of energy supplementation on ingestive behavior during the grazing process, mainly when submitted at the end of grazing period.

Higher rates of substitution for low levels of energy supplementation were reported by Lake et al. (1974), Pordomingo et al. (1991) and Hess et al. (1996), all using corn. In the work of Lake et al. (1974), with cattle grazing orchardgrass with 17 to 20% CP content, it was observed a decrease on forage intake from 2.89% down to 2.57% BW when corn was offered at level of 0.44% BW. Pordomingo et al. (1991) reported decrease on forage intake from 2.76 down to 2.26% BW for grazing animals on temperate grasses with 10.6% CP, and supplemented with whole corn at 0.4%

BW. In the study of Hess et al. (1996) animals grazing fescue with 14.4% CP, had a decrease on forage intake from 3.49 down to 2.82% BW when they were fed cracked corn at 0.34% BW.

Energy supplementation levels around 0.3% BW are more frequent in Brazilian cattle production systems for growing beef cattle on pastures during the wet season, than greater levels, such as 0.6 or 0.9% BW. The high substitution rate observed in this study indicate that low energy supplementation levels such as 0.3% BW, during the wet season, present greater economic risks than higher levels of supplementation. However, in disagreement with the present work, Brokaw et al. (2001), observed a small decrease on forage intake (2.00 vs. 1.92% BW) when animals grazing a 17% CP bromegrass (*Bromus inermis*), were supplemented with cracked corn at 0.34% BW.

According to Bailey (1961), the increases on forage DMI observed for non-supplemented treatments should result in longer rumination time, however, it was not observed in that study as well as in the presented data (Table 9).

### **3.4.3 Rumen Fluid and Blood Samples**

Considering that energy supplement (corn) can provide a potential substrate for propionate production, some authors (MARTIN; HIBBERD, 1990; FALKNER et al., 1994) attributed increases in concentration of propionate and changes in acetate: propionate ratio, to use of supplements. This fact is corroborated by the current study, in which it was observed a significant decrease ( $P < 0.05$ ) for both molar proportion of acetate and acetate: propionate ratio, and increases on propionate molar proportion when 0.3% BW of supplement was fed (Table 12).

Total VFA production, molar proportions of butyrate and ruminal pH were not affected by energy supplementation ( $P > 0.05$ ). However, the ruminal pH was affected by grazing managements, being less for 25 cm (6.39) than for 35 cm (6.52) (Table 12) with average values remaining above 6.2. According to Orskov (1982) and Mertens (1977), microbial growth is not affected until pH drops below 6.2. The results found in this work are corroborated with some reports published in the literature (CATON; DHUYVETTER, 1997; SANTOS et al., 2009) where is emphasized that there is lack of reliable data pointing to rumen pH as the cause of reductions on fiber digestibility, and attributed effects on DMI to other factors.

The N-NH<sub>3</sub> in the rumen fluid of steers in this study decreased linearly ( $P < 0.05$ ) with supplementation (0.3% BW) and grazing management (25 cm pre-grazing sward height) (Table 12). A possible explanation for the supplementation effect is that a higher intake of readily fermentable carbohydrates found in the supplement would provide more substrate for microbial synthesis.

As already mentioned, starch fermentation may influence the rate of utilization of rumen N-NH<sub>3</sub> by changing the energy supply for microbial growth (RUSSELL; MARTIN, 1984). Studies indicate that microbial synthesis is maximized when starch fermentation and proteins degradation are synchronized (FIRKINS, 1996; RUSSELL et al., 1992; BACH et al., 2005). Satter and Slyter (1974) reported that concentrations between 2 and 5 mg N-NH<sub>3</sub>/dL of rumen fluid were required to allow maximum microbial growth. Sampaio et al. (2010) reported 6.24 mg/dL to maximize NDF degradation and Lazzarini et al. (2009) found 15.33 mg/dL to maximize DMI in animals consuming tropical grass (*Brachiaria decumbens*).

Non-supplemented animals had rumen concentration of N-NH<sub>3</sub> in the current study of 11.28 mg/dL. Studies conducted by Detmann et al., 2001, Sales et al., 2008b, Sales et al., 2008a, with tropical grass (*Brachiaria decumbens*) averaging 9.88, 8.75 and 8.97% CP content, respectively, reported levels of N-NH<sub>3</sub> in rumen fluid of 9.88, 11.89 and 10.68 mg/dL, respectively. Franco et al. (2002) with marandu palisadegrass, averaging 10% CP, reported level of N-NH<sub>3</sub> in rumen fluid of 7.3 mg/dL.

The N-NH<sub>3</sub> concentrations were 11.22 and 9.77 mg/dL for 25 and 35 cm pre-grazing sward height (Table 12) and the A+B1 protein fractions were 44.22 and 34.31% (CP basis), respectively (Table 8). It suggests that CP levels and more specifically A+B1 protein fraction may alter the rumen N-NH<sub>3</sub> concentration.

Experimental treatments had no effects on blood glucose ( $P > 0.05$ ). Reed et al. (2007) reported no effects of protein supplementation for steers receiving low-quality hay. Hersom et al. (2004) also did not found differences on blood glucose between non-supplemented and energy supplemented steers.

#### **3.4.4 Microbial Protein Synthesis and Nitrogen Efficiency**

The energy supplementation did not alter ( $P > 0.05$ ) the nitrogen use, when 0.3% of fine ground corn was fed (Table 13). The values of urinary excretion

observed for non-supplemented and supplemented animals was lower (44.73 and 41.89 g/d, respectively) than found in the literature by Nascimento et al. (2010) that worked with *Brachiaria decumbens* and energy-protein supplementation fed at 0.4% BW. The non-supplemented animals used by the latter author consumed approximately 0.894 kg of CP/day and excreted 59.4 g/d of urinary nitrogen. However, the energy-protein supplement (34% of CP) used increased the CP intake to 1.43 kg, what promoted urinary nitrogen excretion of 128.8 g/d. These excretions represent 66 and 56% of nitrogen intake for non-supplemented and supplemented animals (NASCIMENTO et al., 2010), compared to urinary nitrogen excretion at 34 and 33% for non-supplemented and supplemented beef steers in the present study.

It was expected improvements in nitrogen usage and microbial synthesis regarding energy supplementation, most likely because rumen microbes would be able to utilize better the nitrogen present in the forage due to greater quantity of readily fermentable carbohydrates in the rumen (RUSSELL et al., 1992; OWENS; GOETSCH, 1993). However, the high substitution rate, with no increments on digestible DMI (Table 10), limited the mentioned improvements on ruminal fermentation. Several authors (LAZZARINI et al., 2009; FIGUEIRAS et al., 2010; SOUZA et al., 2010) found no increase on microbial synthesis with protein supplementation in tropical conditions, compared with the control treatment, what may suggest energy limitation to improve microbial growth.

These authors found an average 404.68 g/d of microbial synthesis, compared to 434.16 g/d in the present study. The average estimate of microbial efficiency was 120.5 g microbial CP/kg TDN (LAZZARINI et al., 2009; FIGUEIRAS et al., 2010; SOUZA et al., 2010), what is close with suggested by Valadares Filho et al. (2010), and with the presented in the current study (128.8 g microbial CP/kg TDN) (Table 12). This microbial efficiency value is near to recommended values by NRC (1996) and Valadares Filho et al. (2006).

Based on NRC (1996), microbial production can vary from 53 to 140 g microbial CP for each kg of TDN consumed, being used 130 g microbial CP/kg TDN as microbial efficiency.

Even considering negative effects on microbial synthesis when rumen pH is below 6.0 due to damages on cellulolytic bacteria growth (RUSSEL; WILSON, 1996; VERBIC, 2002), in the current study the rumen pH levels observed were above that

value and most likely did not affect the synthesis of microbial protein and consequently NDF digestibility (Table 11 and Table 12).

### **3.4.5 Apparent digestibility and ruminal kinetics**

The supplementation just affected the diet (pasture plus concentrate) DM digestibility, considering the increase of non-fibrous carbohydrate due to energy supplementation (Table 11). In contrast with several papers published in the literature (HANNAH et al., 1989; ZORRILLA-RIOS et al., 1989; VANZANT et al., 1990; PORDOMINGO et al., 1991) that have found reduction on total tract DM and OM digestibilities when grain supplementation was fed, in this current study the corn supplementation at 0.3% BW did not promote reductions on nutrients digestibility, mainly on pastures NDF. This found corroborate with other several studies (LAKE et al., 1974; KRYSL et al., 1989; BRANINE; GALYEAN, 1995; CATON; DHUYVETTER, 1997; SANTOS et al., 2009;), which reported improvements or no effect of energy supplementation on DM, OM or NDF digestibility for cattle consuming forage diets.

The forage protein content may influence strongly the digestibility response to energy supplementation. In situations in which CP is limiting, energy supplementation alone theoretically could worsen the CP deficiency and result in reduced intake, digestibility, and performance (SANSON et al., 1990; CATON; DHUYVETTER, 1997).

However, grazing managements affected diet and pastures nutrients digestibility, what could be related with the nutritional value found in pastures managed with 25 cm pre-grazing sward height (Table 7 and Table 8). The increase on pasture NDF, more expressive on cellulose content (8% of increment) in 35 cm pastures may explain partially the reduction on DMI. The lag time was shorter for the 25 cm compared to the 35 cm pasture (Table 14), probably it is reflect of the less NDF content (Table 7) in pastures with 25 cm treatment. According to Mertens e Loften (1980) the increases in Lag time were responsible to reduce fiber digestion, since the fiber can pass for the posterior tract. Fiber degradation rate was greater for 25 cm pasture. However, due to very similar composition in terms of fiber quality, it was not possible to detect huge changes in fiber digestion either by apparent fiber digestion or by in vitro ruminal kinetics (Table 14). Indeed, pastures managed out of

LI criteria (35 cm pre-grazing sward height) have an important difference on sward structure able to be a limiting factor for forage harvesting.

### 3.5 Conclusions

There is no interaction between low level of energy supplementation and grazing management.

Grazing management is more effective to improve energy intake of grazing cattle than feeding only 0.3% BW of energy supplement.

Grazing management allows cattle not only to increase energy intake but also to decrease energy expenditure with grazing activity.

Corn supplementation fed at 0.3% BW for cattle maintained in a rotational grazing systems reduce grazing time and forage intake of steers grazing well managed (i.e. with adequate levels of CP) Marandu palisadegrass.

Animals supplemented with low levels (0.3% BW) of corn do not have significant increases in energy intake, what may limit responses in weight gains. The low supplementation (0.3% BW) strategy may be riskier with no economic return or even losses because of great substitution effect.

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## 4 FEEDING MEDIUM LEVEL OF ENERGY SUPPLEMENT FOR BEEF CATTLE AND INTERACTIONS WITH GRAZING MANAGEMENT

### Abstract

Eight 24-month-old rumen-cannulated Nellore steers (343 kg BW  $\pm$  7.40) were used in two 4x4 Latin squares to evaluate the interaction between energy supplementation and pre-grazing sward height on cattle grazing behavior, nutrient intake, digestion and metabolism. Treatments corresponded to 0 (mineral supplementation) or 0.6% (0.6% of BW of ground corn as fed basis) combined with 2 pre-grazing sward heights (25 and 35 cm), with a common stubble height of 15 cm for all treatments. Steers were managed on 2 ha of Palisadegrass pasture (*Brachiaria brizantha* cv. Marandu) from February to April of 2011. Pastures were fertilized with 120 kg of nitrogen per ha and averaged 13.8 and 11.0% CP and 58.8 and 63.4% NDF, for the 25 and 35 cm grazing treatments, respectively. The parameters evaluated were forage intake, rumen pH and N-NH<sub>3</sub>, microbial synthesis, VFA, blood glucose, nitrogen balance and grazing behavior. Forage DMI and apparent DM, CP and NDF digestibility were obtained using Cr<sub>2</sub>O<sub>5</sub> and indigestible NDF as digesta markers. Concentration of purine derivative in the urine was used to estimate microbial synthesis. Forage DMI was increased (1.88 vs 1.22%, P<0.05) when pre-grazing sward height was 25 cm and decreased when 0.6% BW supplement was fed (1.77 vs 1.33% BW, P<0.05). The total and digestible DMI were increased by energy supplementation (P<0.05) and when pre-grazing sward height was 25 cm. Steers grazing the 25 cm treatment spent significantly less time grazing and more time resting (P<0.05), took less steps between feeding stations and per day (P<0.05) and presented greater bite rates (P<0.05) when compared with 35 cm treatments. Grazing time was decreased (P<0.05) when supplement was fed, more intensely for animals grazing pastures managed at 35 cm than 25 cm. The DM and CP digestibility of forage and of total diet were greater (P<0.05) for the 25 than the 35 cm grazing management. Energy supplementation increased (P<0.05) diet and forage NDF digestibility and decreased the forage CP digestibility (P<0.05), without effects on CP diet digestibility (P>0.05). Also, the diet DM digestibility was increased feeding 0.6% BW. (P<0.05). Rumen pH (6.50 vs 6.35) was less (P<0.05) and rumen N-NH<sub>3</sub> (12.00 vs 10.01 mg/dL) was greater (P<0.05) for the 25 cm grazing management. Supplementation decreased (P<0.05) rumen N-NH<sub>3</sub>. Microbial synthesis was increased (P<0.05) by energy supplementation. The nitrogen retention was increased (P<0.05) for the 25 cm grazing management and supplementation. Concentration of total VFA was not affected by treatments (P>0.05). However, the energy supplementation decreased rumen acetate and increased propionate (mol/100 mol), resulting in decreased A:P ratio (P<0.05). Blood glucose was not affected by treatments (P>0.05). The pre-grazing sward height of 25 cm and 0.6% BW of energy supplementation were effective to improve harvesting efficiency and energy intake of cattle grazing tropical pasture under rotational grazing.

Keywords: Beef cattle; Energy; Grazing; Management; Supplementation; Tropical grass

## 4.1 Introduction

According to Conrad et al. (1964), forage intake is regulated by the capacity of the digestive tract to digest low quality forage (physical effect or rumen fill) or by the feedback caused by nutrients absorbed from high energy diets (physiological effect). For cattle grazing tropical grasses, once the forage reaches the rumen, the physical regulation is the major mechanism controlling forage intake. However, the forage needs to be harvest before to pass into the gastrointestinal tract and the structure of the sward has a great impact on the efficiency of forage harvesting by grazing cattle, either for temperate grasses (Hodgson et al., 1994) as for tropical grasses (DA SILVA; PEDREIRA, 1996; CARVALHO et al., 2001; DA SILVA et al., 2009). Hodgson et al. (1977) reported that the structure of the sward may have a greater impact on forage intake of grazing cattle than the physical and physiological mechanisms discussed by Conrad et al (1964) and Allen (2000).

Just after the grazing process, the grass initiates its regrowth with intense leaf appearance and leaf growth and minimal stem elongation. The sward will reach a determined height where light will start to become limiting for the lower portion of the sward. Light restriction will stimulate leaves senescence and stem elongation. The result is an increased proportion of senescent material and stems and a decreased proportion of green leaves in the sward, with dramatic alteration on the grazing efficiency and forage intake (DA SILVA; PEDREIRA, 1996; CARVALHO, 2013; SANTOS et al., 2014).

Even in well managed pastures, where the sward structure can improve the grazing efficiency and forage intake, the animal performance is still limited for nutrients intake, what does not permit the expression of the animal's genetic potential. The most limitation is caused by energy intake (DANÉS et al., 2013), due to limitations in forage harvest capacity of the animals and rumen fill caused by fiber content of the tropical grasses (WILSON, 1994) and low particle fragility of grasses (ALLEN, 2000).

The use of concentrate supplementation high in energy is a powerful tool to increase energy intake, stocking rate and cattle performance (VENDRAMINI et al., 2006, 2007; DA CRUZ et al., 2009; FERNANDES et al., 2010). However an interaction between grazing managements and concentrate supplementation is not known. Therefore, the objectives of this study were to evaluate the effects of feeding

an energy supplement at 0 or 0.6% BW and its interaction with grazing management on cattle: 1) ingestive behavior; 2) nutrient intake; 3) rumen parameters; 4) blood glucose; 5) apparent digestibility; 6) rumen fermentation kinetics; and 7) nitrogen use.

## **4.2 Materials and methods**

This article is the second of a set of 2 experiments that evaluated interactions between grazing management and energy supplementation. This article used a medium level of energy supplementation (0.6% BW), while in the companion article a lower level of energy supplementation (0.3% BW) was tested. The experiments had independent animals, but were carried out simultaneously in the same grazing area. Thus, the forage chemical and morphological compositions were the same for both and are presented in the companion article.

### **4.2.1 Experimental area and animals**

The experiment was conducted at the Department of Animal Science of the University of São Paulo (USP), located in Piracicaba-SP, Brazil, during the months of February to April of 2011. The experimental area consisted of 2 ha of marandu palisadegrass managed as a rotational grazing system divided in 16 paddocks and 2 treatments (25 and 35 cm pre-grazing sward height). Eight 24-month-old Nellore steers, 343 kg BW  $\pm$  7.40, cannulated in the rumen were used in this study.

### **4.2.2 Experimental treatments and management practices**

Treatments consisted of 2 levels of energy supplementation (mineral supplementation and 0.6% BW of ground corn as fed basis, combined with 2 pre-grazing sward heights (25 and 35 cm), with a common stubble height of 15 cm for all treatments. Pasture management was performed as a rotational grazing system, with use of nitrogen fertilizer (40 kg/ha per grazing cycle). The targeted pre-grazing sward height of 25 and 35 cm was based on research with marandu palisadegrass correlating these measurements to 95 and 100% light interception (TRINDADE, 2007).

The experiment lasted 64 d and consisted of four experimental periods of 16 d each. Animals were weighed (full BW) prior to the start of each experimental period and the allocation of supplement was fixed for that experimental period. The first 8 d

were used to adapt the animals to the experimental treatments. The following 8 d consisted of sample collections in the following order: on days 9 to 13 the fecal samples collection was performed, on day 14 measurements of animal behavior were taken, on day 15 collections of blood and urine samples were performed and on day 16 rumen fluid samples were collected. Animals had free access to water and minerals supplement in every paddock. The supplemented and the no supplemented experimental animals were brought daily to a management center close to the experimental area where the supplement was in individual feed bunks from 1000h to 1040h. The no supplemented steers were also contained in the feed bunks. This management center was also utilized for infusions of external marker and samplings.

#### **4.2.3 Feed Samples Collection, Chemical Analysis and Calculations**

Forage mass and morphological composition were determined in every grazing cycle, before the steers entered every paddock and after they left. In each evaluation, 3 randomized points were selected in the paddock, and in each point, the material within a rectangle frame ( $0.5 \text{ m}^2$ ) was cut to ground level. Total forage mass was weighted, 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  $55^\circ \text{C}$ . Pre and post-grazing sward height, forage mass, and morphological composition are presented in Table 6.

For chemical composition analysis pasture samples (grazing horizon 15 to 25 and 15 to 35 cm) were cut and collected using a rectangle frame ( $0.5 \text{ m}^2$ ), from 3 distinct points prior to animals' entry in every paddocks for every grazing cycle. Every batch of ground corn was sampled. Feed samples were oven dried at  $55^\circ \text{C}$  for 72 hours and ground through a 1 mm screen (Wiley mill). Pasture samples were bulked as one composite sample by treatment. Corn samples were bulked as one composite sample.

Residual moisture content was determined by drying samples at  $105^\circ \text{C}$  for 24 h. Organic matter content of samples was determined after incineration at  $550^\circ \text{C}$  for 8 h in a muffle furnace (AOAC, 1990). Nitrogen content was determined by the Dumas combustion method (Leco 2000, Leco Instruments Inc) and a conversion factor of 6.25 was used to convert total nitrogen to CP. Contents of NDF, ADF and lignin were

determined according to Van Soest et al. (1991) with no sulphite for all samples analyses and the addition of amylase for supplement analyses only. The NDIN and ADIN were determined using micro Kjeldahl (AOAC, 1990) after digestion of samples with neutral (no sulphite) and acid detergents (VAN SOEST et al., 1991). The TDN values of feed samples were calculated according to Weiss et al. (1992). Forage sample were also analyzed for soluble nitrogen (KRISHNAMOORTY et al, 1982) and for non-protein nitrogen (NPN) (LICITRA et al, 1996) to estimate the protein fractions according to CNCPS (SNIFFEN et al, 1992).

The data regarding pastures chemical and morphological composition are presented in the companion article.

#### **4.2.4 Rumen Fluid and Blood Samples**

Samples of rumen fluid were collected to determine N-NH<sub>3</sub> and VFA concentrations. On day 16 of every experimental period rumen fluid samples (approximately 100 mL) were collected at 0, 2, 4, 6, and 8 h after supplement feeding with the use of a sampling probe (DANÉS et al., 2013). The rumen pH was measured immediately and samples were frozen at -20°C for further analyses. Rumen fluid samples were thawed at room temperature centrifuged (15,000 g, 4°C, 30 min) and analyzed for VFA using gas chromatography (PALMIQUIST; CONRAD, 1971), and for N-NH<sub>3</sub> by phenol-hypochlorite procedure (CHANEY; MARBACH, 1962).

Blood samples were collected on day 15 of the experimental period 4 h after feeding. Blood was collected from the coccygeal vessels 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. Levels of plasma glucose were analyzed by automatic biochemical analyzer YSI 2700 Select.

#### **4.2.5 Forage Intake and digestibility**

Forage intake was calculated from total fecal excretion and the digestibility of feeds. On day 1 and for 13 consecutive days, chromium oxide (Cr<sub>2</sub>O<sub>3</sub>), previously weighed in two paper capsules (5 g each), was dosed into the rumen to all steers,

one dose right after supplement feeding in the morning (1000 h) and a second dose in the afternoon (1700 h). Fecal grab samples (approximately 200 g per animal) were collected between days 9 to 13, twice a day (1000 h and 1700 h), dried at 55°C for 72 h and ground through a 1-mm screen (Wiley mill). Equal amounts of fecal DM 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 (CASALI et al., 2008). The CP and NDF concentrations were analyzed in the feces and multiplied by fecal production, to calculate CP and NDF digestibility.

For 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 RO water (VEGA; POPPI, 1997). The digested samples were analyzed using an inductively coupled plasma spectrometer (ICP-OES).

Forage intake was calculated dividing forage fecal excretion (total fecal excretion minus the amount resultant from the supplement) by the forage indigestibility.

#### **4.2.6 Animal Behavior**

Grazing behavior measurements took place on d 14 of each period. Four trained observers were assigned to visually determine grazing (eating plus searching), rumination, and idling time every 5 min for consecutive 24 hours. The number of observations for each activity during a 24 hours period was multiplied by 5 minutes to get the daily amount of time spent in that activity. While grazing, cattle search, acquire into the mouth, masticate, and swallow herbage (Gibb, 1998). The observers also counted bite rate (bites/min) using the time spent to 20 bites (PENNING; RUTTER, 2004).

Each eating step was considered a feeding station (RUYLE; DWYER, 1985; ROOK et al., 2004). The feeding stations per minute were calculated considering the time spent to 10 feeding station divided by 10. Also, it was measured the number of steps between feeding stations, and then it was calculated the total steps per day, multiplying the steps per minute by grazing time (min).

#### 4.2.7 Microbial Protein Synthesis and Nitrogen Efficiency

Nitrogen intake was calculated by the sum of intakes of N from forage and supplement origins. Apparent efficiency of N use (assuming no retention or mobilization of N in the body) was calculated for every steer by dividing mean retained N (N intake minus N in urine and feces) by the average N intake.

Microbial synthesis was estimated through the excretion of purine derivatives (STANGASSINGER et al., 1995). Spot urine samples were collected on day 15 of each experimental period, in a single sample collected approximately 4 h after supplement feeding. Sub-samples of urine (10mL) 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 a HPLC (PIMPA; BALCELLS, 2002), and for N using micro Kjeldhal (AOAC, 1990).

Total urine production was estimated using the creatinine content in the sample, assuming that excretion of creatinine is constant (0.213 mmol kg BW<sup>-1</sup>) (CHIZZOTTI et al. 2008). The excretion of purine derivatives (PD) was then calculated as the sum of allantoin and uric acid excreted in urine, expressed in mmol/d. The analyses of PD were performed according to Pimpa et al. (2001) using values of 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 e Gomes (1992).

#### 4.2.8 Rumen Fermentation Kinetics

The *in vitro* rumen fermentation analysis were described and discussed in the companion article because the incubated feed samples were the same for both experiments.

#### 4.2.9 Statistical Analysis

The experimental design was a double 4x4 Latin Square design, with eight animals, four treatments and four periods. All variables, except the rumen fermentation kinetics and its predicted parameters, were analyzed using the PROC MIXED package of SAS statistical system (SAS<sup>®</sup> version 9.2<sup>®</sup>, 2008). The rumen fermentation kinetics variables were analyzed in a completely randomized design using PROC GLM package of SAS statistical system (SAS<sup>®</sup> version 9.2<sup>®</sup>, 2008). The

model included treatment, animal and period as sources of variation to analyze the data of forage, nutrient intake, animal behavior, N excretion, blood parameters and microbial synthesis. Treatments and periods were considered fixed, while animal and overall errors were considered random effects. The model used to analyze rumen parameters (pH and concentrations of VFA and N-NH<sub>3</sub>) collected at different times after feeding, included treatment, period, animal, hours post-feeding as repeated measures and treatment x hours post-feeding interaction as sources of variation.

All variables were considered fixed, except animal, whole-plot error, and subplot error, which were considered random. The covariance structure used for repeated measures analyses was chosen based on Bayesian Information Criteria parameter of SAS statistical system (SAS<sup>®</sup> version 9.2<sup>®</sup>, 2008) closer to zero. The averages were calculated by Least squares means and adjusted for comparison by Tukey test at 5% probability. The averages were calculated by Least squares means and adjusted for comparison by Tukey test at 5% and 10% probability. Thus, significance was declared at  $P < 0.05$  and  $P < 0.01$ .

The parameters of rumen fermentation kinetics predicted by the equations were fitted by nonlinear models using PROC NLIN of the SAS statistical system (SAS<sup>®</sup> version 9.2<sup>®</sup>, 2008). Necessarily, all data sets were tested before the final overall analysis to ensure that all the assumptions of analysis of variance (additive model, independence of errors, data normality and homoscedasticity) were met.

## **4.3 Results**

### **4.3.1 Pasture and Supplement Samples**

The data regarding pastures managements were presented in the companion article.

### **4.3.2 Forage Intake and Animal Behavior**

There were interactions between grazing management and energy supplementation (Table 15) for grazing time ( $P < 0.05$ ), for rumination time ( $P < 0.1$ ), for steps between feeding stations ( $P < 0.1$ ) and for steps per days ( $P < 0.10$ )

When 0.6% BW of corn was fed for animals grazing pastures managed with 25 cm of sward height, grazing time was reduced in 22 minutes ( $P < 0.05$ ), but for

pastures managed with 35 cm, grazing time was reduced 120 minutes ( $P<0.05$ ) (Figure 1).

However, this reduction on steps per day and steps between feeding stations were more intense in animals maintained in pastures managed at 35 cm of pre-grazing sward height (Figure 2 and Figure 3), as shown by the significant ( $P<0.10$ ) interaction between grazing management and energy supplementation presented in the Table 15.

Animals grazing pastures managed with 25 cm sward height decreased the ruminantion time ( $P<0.05$ ) to 400 min/d for 372 min/d, when 0.6% BW as fed. However, when the 0.6% BW was fed for animals grazing pastures managed with 35 cm sward height there was no difference ( $P>0.05$ ) in ruminantion time in comparison with non-supplemented animals (488 and 496 min/d for 0 and 0.6% BW, respectively).

Animals kept on pastures managed with 25 cm, presented more time resting and greater bite rates ( $P<0.05$ ) compared to cattle grazing pasture with 35 cm sward height as the start grazing point (Table 15).

The number of feeding stations per minute was greater ( $P<0.10$ ) for animals grazing pastures with 25 cm. The steps per minutes were not affected by grazing management ( $P>0.05$ ). However, as consequence of greater grazing time for 35 cm treatment the total steps per day also was greater ( $P<0.05$ ) for 35 cm pre-grazing sward height (Table 15).

The energy supplementation had no effect ( $P>0.05$ ) on resting time, bite rates and feeding stations but it increased ( $P<0.05$ ) steps per minute.

Table 15 - Effects of levels of energy supplementation and grazing management on grazing behavior

	Management, cm		Level of supplementation, % BW		P value			SEM
	25	35	0	0.6	M	S	M*S	
Grazing time, min/d	390.05	434.61	447.80	376.80	0.0251	0.0011	0.0152	29.23
Ruminating time, min/d	392.06	441.66	401.66	431.97	0.2416	0.4687	0.0870	36.43
Rest time, min/d	607.72	502.28	543.49	566.51	0.0143	0.5011	0.6450	35.05
Bit rate, bites/min	29.72	19.59	23.41	25.90	0.0360	0.5980	0.5025	3.69
Feeding stations/min	5.17	4.12	4.75	4.55	0.0615	0.7112	0.4503	0.56
Steps between FS	1.14	1.62	1.54	1.22	0.0002	0.0082	0.0623	0.10
Steps/min	5.96	6.52	6.97	5.50	0.3580	0.0253	0.6137	0.64
Steps/day	2244.1	2912.7	3089.4	2067.8	0.0186	0.0010	0.0791	266.6

<sup>†</sup>Standard error of the means, M=management, S=supplementation, M\*S=interactions between management and supplementation.

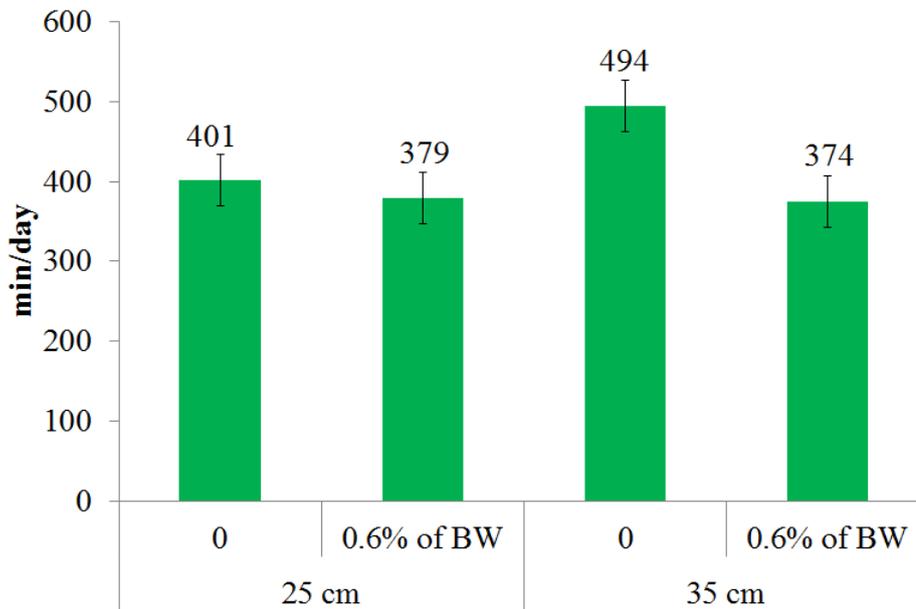


Figure 1 - Interactions between grazing management and energy supplementation on grazing time

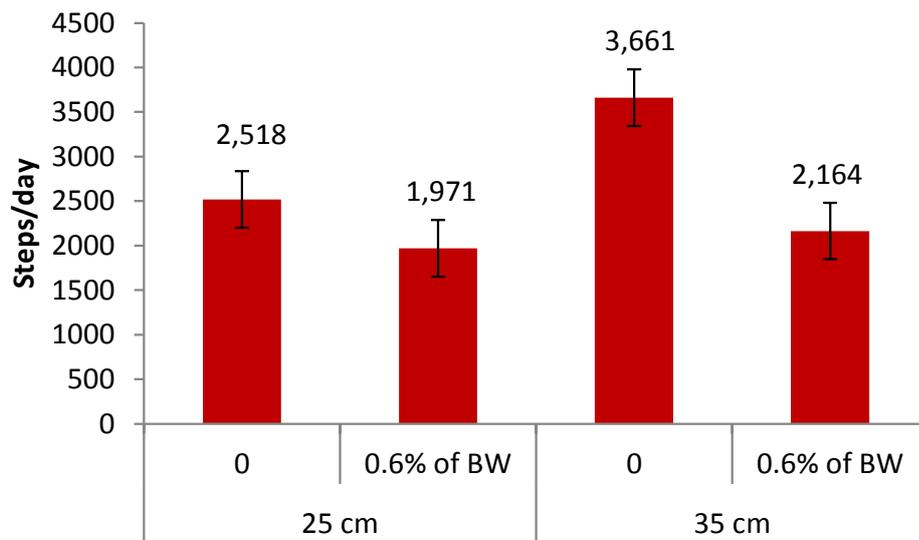


Figura 2 - Interactions between grazing management and energy supplementation on steps per day

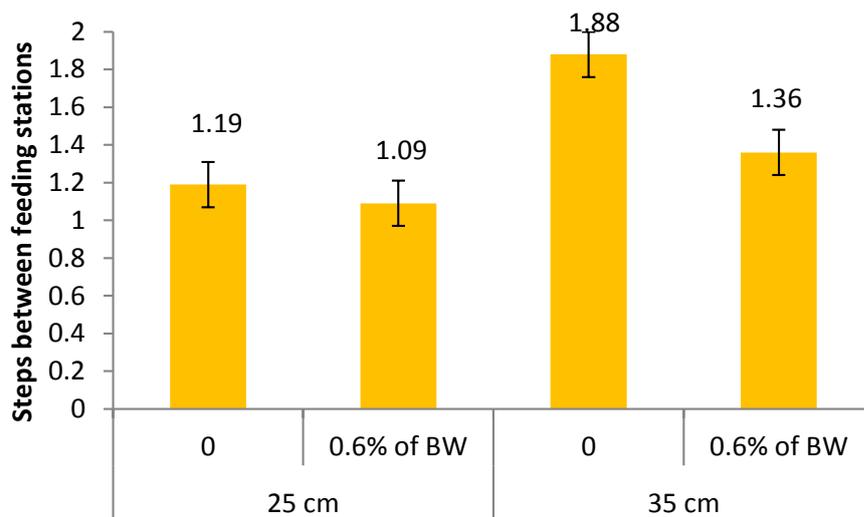


Figura 3 - Interactions between grazing management and energy supplementation on steps between feeding stations

There was no interaction between grazing managements and level of energy supplementation ( $P>0.05$ ) for feed intake. Cattle grazing pastures with 25 cm (correlated to 95% LI) had greater intakes of forage, of total DM, of digestible DM, of NDF and of CP ( $P<0.05$ ) than animals grazing pastures managed with 35 cm as start grazing point (Table 16). Energy supplementation reduced forage and NDF intakes ( $P<0.05$ ), with no effect on CP intake ( $P>0.05$ ) (Table 16). The total DM and energy intake were increased when 0.6% BW of corn was fed. The substitution rate for feeding 0.6% BW of ground corn was 0.72 ( $P<0.05$ ).

Table 16 - Effects of energy supplementation and grazing management on forage, total, digestible, NDF and CP intakes and substitution rate

	Management, cm		Level of supplementation, % BW		P value			SEM
	25cm	35cm	0	0.6	M	S	M*S	
Forage DMI, % BW	1.88	1.22	1.77	1.33	0.0032	0.0385	0.7159	0.31
Total DMI, % BW	2.18	1.52	1.77	1.93	0.0030	0.0430	0.7155	0.33
Digestible DMI, % BW	1.47	0.99	1.05	1.36	0.0013	0.0251	0.3416	0.17
NDF DMI, % BW	1.14	0.81	1.07	0.87	0.0163	0.0961	0.6350	0.20
CP DMI, % BW	0.29	0.16	0.22	0.22	0.0001	0.9234	0.9432	0.04
Substitution rate	0.00	0.00	0.00	0.72	0.4734	0.0030	0.4921	0.27
Forage DMI, kg/d	5.40	3.60	5.19	3.81	0.0034	0.0188	0.7027	0.77
Total DMI, kg/d	6.31	4.51	5.20	5.62	0.0027	0.0438	0.6914	0.74
Digestible DMI, kg/d	4.28	2.95	3.24	3.99	0.0011	0.0452	0.5964	0.41
NDF DMI, kg/d	3.27	2.38	3.15	2.50	0.0118	0.0546	0.6155	0.46
CP DMI, kg/d	0.83	0.48	0.66	0.66	0.0001	0.9965	0.9438	0.10

<sup>1</sup>Standard error of the means, <sup>2</sup>= kg of reduction on forage DMI for kg of supplemented fed, M=management, S=supplementation, M\*S=interactions between management and supplementation

#### 4.3.3 Apparent digestibility

The forage DM and the forage and diet CP digestibility were greater ( $P < 0.05$ ) for 25 cm pre-grazing sward height than for 35 cm, while forage NDF, diet NDF and diet DM were not affected ( $P > 0.05$ ) by pasture management (Table 17). Energy supplementation increased forage NDF digestibility ( $P < 0.05$ ) and decreased ( $P < 0.05$ ) forage CP digestibility, with no negative effects ( $P > 0.05$ ) on forage DM digestibility. When corn was fed at 0.6% the diet DM digestibility was increased ( $P < 0.05$ ) as well as the diet NDF digestibility ( $P < 0.10$ ), with no effects on CP digestibility (Table 17).

Table 17 - Effects of energy supplementation and grazing management on forage and diet nutrients digestibility

	Management, cm		Level of supplementation, % BW		P value			SEM
	25cm	35cm	0	0.6	M	S	M*S	
Forage digestibility (%)								
DM	62.94	57.64	61.70	58.90	0.0001	0.1530	0.1652	1.44
CP	71.41	58.71	69.91	60.21	0.0001	0.0005	0.1311	2.39
NDF	65.15	64.36	62.71	66.8	0.5756	0.0077	0.7914	1.27
Diet digestibility (%)								
DM	68.03	65.74	61.83	71.94	0.2752	0.0001	0.8657	1.77
CP	74.53	66.33	70.03	70.83	0.0017	0.7331	0.7191	2.16
NDF	64.48	63.39	62.68	65.19	0.4408	0.0823	0.9495	1.19

<sup>1</sup>Standard error of the means, M=management, S=supplementation, M\*S=interactions between management and supplementation

#### 4.3.4 Rumen Fluid and Plasma Glucose

There was no interaction between grazing management and energy supplementation ( $P>0.05$ ) for rumen pH, VFA, and plasma glucose (Table 18).

However, there was interaction between energy supplementation and grazing management for N- N-NH<sub>3</sub>. The rumen N-NH<sub>3</sub> was reduced more intense feeding 0.6% BW of energy supplementation for animals grazing pastures managed with 35 cm sward height (Figure 4).

The rumen pH was less ( $P<0.1$ ) for cattle grazing the 25 cm sward height and for cattle supplemented with ground corn at 0.6% BW ( $P<0.05$ ). The rumen N-NH<sub>3</sub> was greater ( $P<0.05$ ) for cattle grazing the 25 cm pasture and less ( $P<0.05$ ) for cattle supplemented with ground corn at 0.6% BW. Total VFA concentration was not affected ( $P>0.05$ ) by treatments, but supplementing energy increased rumen propionate proportion ( $P<0.05$ ) and decreased rumen acetate and acetate:propionate (A:P) ratio ( $P<0.05$ ). Plasma glucose was not affected by increasing energy supplementation and grazing management ( $P>0.05$ ).

Table 18 - Effects of energy supplementation and grazing management on rumen pH, NH<sub>3</sub>/N, volatile fatty acids (VFA) and plasma glucose

	Management, cm		Level of supplementation, % BW		P value			SEM
	25	35	0	0.6	M	S	M*S	
pH	6.39	6.46	6.50	6.35	0.0854	0.0001	0.7149	0.14
N-NH <sub>3</sub> ,mg/dL	12.00	10.01	12.62	9.38	0.0061	0.0001	0.0682	1.19
Total VFA,mM	142.83	135.14	135.13	142.84	0.3597	0.3585	0.3615	11.88
		mol/100 mol						
Acetate	68.85	68.83	70.69	66.99	0.9854	0.0001	0.3378	0.78
Propionate	20.89	20.50	18.90	22.48	0.6214	0.0001	0.1455	0.81
Butyrate	10.61	11.17	11.10	10.67	0.2556	0.3858	0.6490	0.35
A:P	3.48	3.64	3.94	3.18	0.2681	0.0001	0.1915	0.14
Plasma glucose, mg/dL	55.34	54.87	56.13	55.83	0.8723	0.7634	0.3185	1.76

<sup>1</sup>Standard error of the means, M=management, S=supplementation, M\*S=interactions between management and supplementation

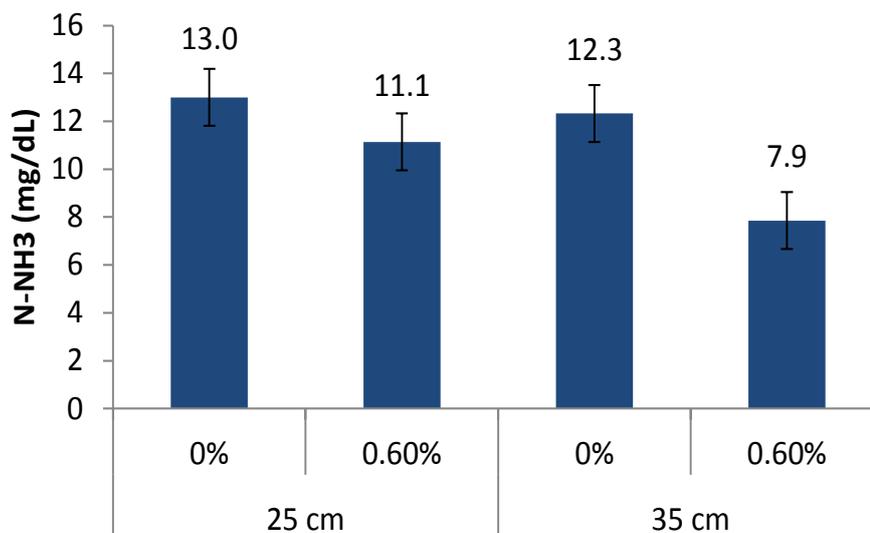


Figure 4 - Interactions between grazing management and energy supplementation on N-NH<sub>3</sub>

#### 4.3.5 Microbial Protein Synthesis and Nitrogen Efficiency

There was no interaction ( $P>0.05$ ) between energy supplementation and grazing management for nitrogen use and microbial synthesis (Table 19). The energy supplementation increased ( $P<0.05$ ) the nitrogen retention, efficiency of N use and microbial protein synthesis of cattle grazing fertilized Palisadegrass pasture. Managing the pasture with 25 cm pre-grazing sward height caused a huge increase

( $P < 0.05$ ) in the intake of N with greater retention of N ( $P < 0.05$ ) and greater efficiency of N utilization ( $P < 0.05$ ) by cattle grazing fertilized tropical pasture.

Table 19 - Effects of energy supplementation and grazing management on nitrogen intake, nitrogen urine, nitrogen feces, nitrogen retained (% N intake), microbial protein (MicP) and microbial efficiency (MicEf)

	Management, cm		Level of supplementation, % BW		<i>P value</i>			SEM <sup>1</sup>
	25	35	0	0.6	M	S	M*S	
N fecal <sup>2</sup>	39.94	36.56	38.12	38.38	0.6142	0.9688	0.9688	7.24
N urinary <sup>2</sup>	41.51	33.22	40.73	34.00	0.0205	0.0553	0.2951	2.43
N excretion <sup>2</sup>	80.93	69.78	78.56	72.14	0.1202	0.3575	0.4936	7.76
N intake <sup>2</sup>	135.03	98.68	112.77	120.95	0.0129	0.5442	0.9895	14.37
N retention <sup>2</sup>	53.94	29.94	35.15	48.73	0.0205	0.1646	0.5826	8.65
N retention, %N intake)	39.80	30.32	30.16	39.28	0.0463	0.0477	0.8794	3.10
MicP <sup>2</sup>	401.59	376.83	357.07	421.35	0.3842	0.031	0.4118	24.47
MicEf. g/kg of TDN	108.22	134.80	124.41	118.61	0.0941	0.7026	0.5387	16.38

<sup>1</sup>Standard error of the means, <sup>2</sup>g/day, M=management, S=supplementation, M\*S=interactions between management and supplementation

## 4.4 Discussion

### 4.4.1 Animal Behavior and Forage Intake

As discussed in the companion article, the high fiber content and low particle fragility of tropical forages are the main factors that promote rumen fill effect, being responsible to limit forage intake in grazing animals (STOBBS, 1973; POPPI et al., 1980, 1981a, 1981b, 1985; ALLEN, 200). However, the sward structure also imposes limitations on forage intake, as demonstrated in this study.

The effect of grazing management on DMI was the same discussed in the companion article, where the adoption of a start grazing point based on a sward height (25cm) correlated with 95% light interception for marandu Palisadegrass promoted increases on DMI (total, forage, digestible, NDF and CP) as response to

decreases on harvesting efficiency (Table 15). The greater proportions of stems and senescent material in the 35 cm pasture contributed to reduce harvest efficiency during the grazing down process acting as a horizontal barrier (UNGAR et al., 2001; BENVENUTTI et al., 2006, 2009; DRESCHER et al., 2006; BAGGIO et al., 2009).

In the companion article were observed reductions in bite rate of 34% when animals were managed in pastures with 35 cm in comparison with 25 cm of initial sward height. The same value (34%) was observed in the present study, indicating a consistent impact of sward height and structure on grazing behavior (CARVALHO 1997; BAGGIO et al., 2009).

In the current study was aimed to increase the amount of ground corn supplementation (0.6% BW) to evaluate its interaction with grazing management. Thus, when the level of corn was 0.6% BW, there was interaction between grazing management and energy supplementation for grazing time.

In both grazing management the grazing time was reduced when cattle were supplemented. However, for the 25 cm pastures animals fed corn at 0.6% BW grazed 22 minutes less while for the 35 cm pastures, feeding corn decreased grazing time in 120 minutes (Figure 1). This fact indicates that in pastures where the sward structure is not adequate, the supplementation may cause greater substitution rate than in pastures with more favorable sward structure. This may limit the increment in energy intake caused by supplementation in not well managed pastures.

The energy supplementation affected the number of feeding stations per minute ( $P < 0.05$ ), where supplemented animals had less feeding stations per minute than non-supplemented animals. It means that the supplemented animal spent more time per feeding station, what suggests that supplemented animals may select more and use better each feeding station (Table 15). Non-supplemented animals need to harvest more due to energy status and it reflects on the number of feeding stations. According to several authors (THOMSON et al., 1985; GREGORINI et al., 2007, 2009) the level of hunger influences forage intake rate and grazing dynamics.

The combination between greater bite rates and less grazing time suggests greater harvesting efficiency, what may be confirmed by greater DMI (Table 16) for animals grazing pastures with 25 cm of initial sward height. Also, this information suggests less energy expenditure for grazing activities, for the 25 cm treatments, supported by all mentioned parameters, mainly by greater resting time.

Animals grazing pastures managed at 35 cm spent more time per feeding station (Table 15), suggesting that the greater forage mass and the time to manipulating the forage before to eat, may have influenced the time spent per feeding station (BAGGIO et al., 2009). The number of steps between feeding stations and steps per day found for 35 cm treatments showed that animals walked more searching for better feeding stations. The forage allowance at each feed station determines the time spent by the animal at the feeding station (BAGGIO et al., 2009).

This result corroborates with some authors that found effects of supplementation on displacement patterns, where non-supplemented beef heifers traveled greater distances and visited more feeding stations per minute, while the opposite happened for supplemented animals (GLIENKE et al., 2010). A hypothesis raised was that supplemented animals spent less time searching for feeding stations because they had their nutritional requirements met. In the other hand, other study reported that supplemented animals were more selective and covered greater distances in search for better feeding stations (ADAMS, 1985).

The interaction between grazing managements and energy supplementation for steps between feeding stations and steps per day is showed in the Figure 2 and Figure 3. In both interactions is possible to observe that the impact of energy supplementation was greater in animals maintained in pastures managed at 35 cm of sward height, corroborating whit the reported for grazing time. Even without detect interactions for forage DMI and substitution rate, the behavioral parameters suggest that the substitution rate could have been greater for supplemented animals grazing pastures managed with 35 cm as the start grazing point.

Feeding ground corn at 0.6% BW affected forage DMI, total and digestible DM for both 25 and 35 cm grazing managements (Table 16). The interaction observed for grazing time was not observed for forage intake.

Energy supplementation decreased forage DMI as consequence of substitution effect, but it increased total and digestible DMI (Table 16), differently of the observed in the companion article, where 0.3% BW was not able to increase digestible DM. Dórea (2011) also reported that feeding ground corn at 0.3% BW for cattle on tropical pastures under rotational grazing failed to increase energy intake. Correia (2006), fed 0, 0.3, 0.6 and 0.9% BW of an energy supplement for cattle on a high stocked pasture of Palisade grass under rotational grazing. The 0.3% level had

slight effect on cattle ADG and a great impact on pasture stocking rate compared to the no supplemented treatment.

Feeding 0.6% BW or greater was very effective to increase ADG and beef production per area. Costa (2007) and Dell Agostinho Neto (2010) also reported that feeding ground corn at 0.6% BW was a confident level to increase cattle performance, pasture stocking rate and beef production per area.

#### **4.4.2 Rumen Fluid and Blood Samples**

The energy supplementation did not increase the total VFA as supported by other researches with readily fermentable fiber and starch sources (MARTIN; HIBBERD, 1990; CAREY et al., 1993). However the molar proportions of acetate was lower ( $P < 0.05$ ) in supplemented steers than in non-supplemented steers. Conversely, molar proportions of propionate were greater ( $P < 0.05$ ) for supplemented steers than for no supplemented steers (Table 6). As a result, the acetate:propionate was lower for supplemented steers. Thus, cattle supplemented with corn (0.6%BW) even without an increase in total VFA concentration had more propionate available for metabolism than no supplemented cattle (Table 18). Increased propionate and decreased acetate as a result of concentrate inclusion in the diet is well documented (HORN; McCOLLUM, 1987). Butyrate molar proportions did not change for any treatments. Butyrate proportions have been documented to increase with increasing starch consumption (STERN et al., 1978; GRIGSBY et al., 1991).

The greater intake of higher quality forage of cattle on the 25 cm pastures resulted in lower ruminal pH (6.39 vs 6.42) compared to the 35 cm pastures. Feeding 0.6% of ground corn also decreased rumen pH (6.35 vs 6.50) compared to no supplementation. However even for supplemented cattle rumen pH remained always above 6.2 (Table 18). According to Mertens (1977) the forage fiber digestion declines when ruminal pH fell below 6.7. Later, Ørskov (1982) and Mould et al. (1983) indicated that ruminal pH below 6.2 would reduce the activity of cellulolytic bacteria and digestion of straw, respectively. These researchers indicated that depressions in ruminal pH could be responsible for reductions in forage fiber digestibility associated with grain supplementation. Some studies indicated that populations of cellulolytic bacteria were reduced in pH ranges from 5.7 to 6.2, whereas soluble carbohydrate fermenting bacteria persist until ruminal pH ranges from 4.6 to 4.9 (RUSSELL et al., 1979; RUSSELL; DOMBROWSKI, 1980) Sensitivity of ruminal bacteria to pH and

shifting in the bacterial populations in response to reduced pH have been suggested as reasons for reduced forage digestion and consequent reduced forage intake by grazing ruminants fed high energy supplements (HORN; McCOLLUM, 1987). However, the results in this work are corroborated with some reports published in the literature (CATON; DHUYVETTER, 1997; SANTOS et al., 2009) where is emphasized that there is lack of reliable data pointing to rumen pH as the cause of reductions on fiber digestibility, and attributed effects on DMI to other factors.

As found in the companion article the N-NH<sub>3</sub> in the rumen fluid of steers was reduced in two different ways: a) due to energy supplementation (0.6% BW) (12.62 vs 9.38 mg/dL for 0 and 0.6% BW of energy supplementation, respectively); b) due to grazing management (12.00 vs 10.01 mg/dL for 25 and 35 cm pre-grazing sward height, respectively) (Table 18).

However, due to lower CP content and protein fractions characteristics for pastures managed with 35 cm sward height, when 0.6% of energy supplementation was fed, the N-NH<sub>3</sub> in the rumen fluid was reduced more intense, in comparison with animals maintained in pastures managed with 25 cm sward height. Its suggests that even with lower levels of N-NH<sub>3</sub> in non-supplemented animals kept in 35 cm grazing management, the utilization of this N-NH<sub>3</sub> by rumen microorganism were greater than in animals grazing pastures with 25 cm sward height, resulting in the more intense N-NH<sub>3</sub> reduction observed.

The greater intake of readily fermentable carbohydrates may explain the supplementation effect, what provided more substrate for microbial synthesis (RUSSELL; MARTIN, 1984) as can be observed in Table 18. According to Grigsby et al. (1991), the reduction of ruminal N-NH<sub>3</sub> in steers supplemented with soybean hulls was attributed to increased utilization of N-NH<sub>3</sub> for microbial protein synthesis. It is reported in the literature that microbial synthesis is increased when starch and proteins degradation are synchronized (RUSSELL et al., 1992; FIRKINS, 1996; BACH et al., 2005).

As reported in the companion article, the pastures managed at 25 cm of sward height contained greater CP content and also had A+ B1 fraction 28% higher than pastures managed at 35 cm pre-grazing sward height, what may explain the highest N-NH<sub>3</sub> concentrations in pastures managed at 25 cm sward height.

There was no effect of any treatments on blood glucose ( $P > 0.05$ ), what is corroborated by data from Reed et al. (2007) and by Hersom et al. (2004).

#### 4.4.3 Microbial Protein Synthesis and Nitrogen Efficiency

Feeding ground corn at 0.6% BW improved the utilization of dietary N (Table 19). This improvement is explained by more rapidly fermentable carbohydrate in the rumen, what reduced rumen N-NH<sub>3</sub>, and increased microbial synthesis (RUSSELL et al., 1992; OWENS; GOETSCH, 1993), and also because of more of the absorbed N is incorporated in animal tissues. Animals fed corn at 0.3% BW did not reduce N in urine neither increased N retention, as showed in companion article and reported by Dorea (2011), because energy intake was not increased. For cattle grazing tropical forages with 5.08, 7.55 and 5.16 % CP (LAZZARINI et al., 2009; FIGUEIRAS et al., 2010; SOUZA et al., 2010) it was not observed improvements on microbial synthesis when protein supplements were fed, suggesting that energy was limiting the increases on microbial growth.

Improving pasture management was an effective way to increase protein intake, due to greater CP content of forage and greater forage intake. Even so, cattle grazing the 25 cm pastures succeeded to excret less N in the urine and to retain more N than cattle grazing the 35 cm pastures, because of greater energy intake.

The average estimate of microbial efficiency in the current study was 121.5 g microbial CP/ kg TDN. This value is close to the average found in the literature (120.5 g microbial CP/kg TDN) by Lazzarini et al. (2009), Figueiras et al. (2010), Souza et al. (2010). The average found in the literature as well as in this current study is close with suggested by Valadares Filho et al. (2010), but is a little lower than suggested by the NRC (1996) (Table 19).

#### 4.4.4 Apparent digestibility

Curiously the energy supplementation did not increase forage DM digestibility, but increased the NDF digestibility (Table 17). The forage CP digestibility was decreased with the energy supplementation and this fact can be explained by the increase in microbial synthesis. This results in less N being excreted via urine and more N excreted in feces.

Twelve articles published in the literature (MARTIN; HIBBERD, 1990; CAREY et al., 1993; FAULKNER et al., 1994; ELIZALDE et al., 1998; DETMAN et al., 2001; LARDY et al., 2004; RICHARDS et al., 2006; PAVAN; DUCKET, 2008; SALES et al., 2008a, 2008b; NASCIMENTO et al., 2010, 2009) were compiled and a meta-analysis

was run to test the effect of concentrate supplementation on NDF digestion on total tract. There was not effect on NDF digestion, unlike reported on a few papers published in the literature (HANNAH et al., 1989; ZORRILLA-RIOS et al., 1989; VANZANT et al., 1990; PORDOMINGO et al., 1991).

The NDF digestibility in the total tract found in this present study (65.19%) (Table 17) is close to observed in the meta-analysis intercept (64.00%). This findings corroborate with other several studies (LAKE et al., 1974; KRYSL et al., 1989; BRANINE; GALYEAN, 1995; CATON; DHUYVETTER, 1997; SANTOS et al., 2009;), which reported improvements or no effect of energy supplementation on DM, OM or NDF digestibility for cattle consuming forage diets.

Pastures managed with 25 cm produced forage with greatest DM digestibility. The major difference in chemical composition between the 25 and the 35 cm pastures was the content of NDF. However, for both pastures, NDF digestibility was greater than DM digestibility and the digestibility NDF was not different between the pastures. Lopes (2011) reported that FDN from well managed tropical grasses presented greater in vitro digestibility than alfalfa silage.

#### **4.5 Conclusions**

For cattle grazing *Brachiaria Braizantha* cv. Marandu, the adoption of 25 cm of sward height as the start grazing point is an effective tool to increase energy intake and decrease energy expenditure with grazing activity.

Feeding ground corn at 0.6% BW is an effective tool to increase energy intake and to decrease energy expenditure with grazing activity.

Substitution of grain for forage may be greater when pastures are not well managed in rotational grazing systems.

Improving pasture management and feeding corn at 0.6% BW are effective tools to improve forage CP utilization.

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