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MARANDU GRASS MANAGEMENT AND SUPPLEMENTATION ON PERFORMANCE AND METHANE MITIGATION OF NELLORE BULLS

Andre Luis da Silva Valente

Zootecnista

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Orientador: Prof. Dr. Ricardo Andrade Reis

Tese apresentada à Faculdade de Ciência Agrária e Veterinária – Unesp, Câmpus de Jaboticabal, como parte das exigências para obtenção do titulo de doutor em zootecnia.

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CERTIFICADO DE APROVAÇÃO

TÍTULO: MARANDU GRASS MANAGEMENT AND SUPPLEMENTATION ON PERFORMANCE AND METHANE MITIGATION OF NELLORE BULLS

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CURRICULUM

Andre Luis da Silva Valente was born in Anapolis, Goias, Brazil, on September 21, 1986. Graduated in Animal Science by Goias State University at Sao Luis de Montes Belos campus, Goias, Brazil in 2008. In March of 2009 he started his M.Sc. program in Animal Science at the Sao Paulo State University, under the guidance of Prof. Dr. Ricardo Andrade Reis, working with forage management and animal production on pasture. He received his M.Sc. degree in February of 2011. In March of 2011 he started his PhD degree in Animal Science at the same University. During his program he worked with forage and ruminant production on pasture and feedlot. The mainly aim of this research were assess new strategic to optimize the beef cattle system and mitigation of greenhouse gases emission. He arrived at Range Cattle REC - University of Florida, USA in March 2011 as an exchange visitor under advising of Prof. Dr. Joao Vendramini where worked with beef cattle production until November of 2011. In October of 2013, Andre went to The University of Queensland – Gatton campus for a new exchange visitor under advising of Prof. Dr. Dennis Poppi. During his time in Australia the mainly research was assess the effect of nutrition on skeletal growth and its relationship with cattle performance.

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SUMMARY

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Certificamos que o Protocolo nº 021119/11 do trabalho de pesquisa intitulado **"Balanço de gases de efeito estufa e estratégias de mitigação em pastos de Brachiaria submetidos a diferentes manejos"**, sob a responsabilidade da Profª. Drª. Telma Teresinha Berchielli está de acordo com os Princípios Éticos na Experimentação Animal, adotado pelo Colégio Brasileiro de Experimentação (COBEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 07 de Outubro de 2011.

Jaboticabal, 11 de Outubro de 2011.

Prof. Dr. Jeffrey Frederico Lui
Presidente - CEUA

Med. Vet. Maria Alice de Campos
Secretária - CEUA

MANEJO DE PASTAGEM E SUPLEMENTAÇÃO NO DESEMPENHO E MITIGAÇÃO DE METANO DE BOVINOS NELORE

Resumo

O correto manejo do pastejo e o uso estratégico da suplementação são os principais caminhos quando se visa aumentar a produção animal e/ou o ganho de área no sistema a pasto. No entanto, dados divergentes sobre o correto manejo em forrageiras tropicais e o efeito da suplementação com soja diferente disponível no rúmen ainda são encontrados. Nesse sentido, três experimentos foram realizados para avaliar o efeito da suplementação de touros jovens da raça Nelore em pastagem de Brachiaria brizantha cv. Marandu manejado em diferentes alturas. No primeiro experimento os tratamentos consistiram de três alturas do dossel (15, 25 e 35 cm) do pasto e foram estudadas as características de forragem, consumo de matéria seca, digestibilidade, desempenho animal e ganho por área durante a estação chuvosa. Foram utilizados trinta e seis animais tourinhos Nelore. Aumentar a altura do dossel refletiu em aumento da massa de forragem, enquanto diminuiu a taxa de lotação. Não houve diferença na composição química de PB, FDN e concentração DIVMS entre os tratamentos. No segundo experimento, foi avaliado o efeito da suplementação (sal mineral; suplemento com gordura protegida; suplemento com soja grão) no desempenho e produção de metano de bovinos mantidos em pastos de *Brachiaria brizantha* cv. Marandu manejados a 25 cm. Animais suplementados apresentaram valores de consumo e digestibilidade superior para todos os nutrientes, exceto proteína quando comparado ao tratamento controle. No entanto, a suplementação não afetou a produção de CH4, expressos em g CH4 / dia. O aumento no ganho de peso vivo dos animais suplementados refletiu em diminuição na produção de CH_4 / kg de produto e g CH_4 / kg CMS. No terceiro experimento foi avaliado um fatorial 3x3 - três alturas do dossel (15, 25 e 35 cm) e três suplementos (sal mineral; suplemento com gordura protegida; suplemento com soja grão) no desempenho, consumo e digestibilidade de bovinos mantidos em pastos de capim marandu. Não foram encontradas interações entre a altura e suplemento. O aumento da altura refletiu em aumento do desempenho animal enquanto que animais suplementados com gordura protegida apresentaram o maior desempenho. Com base nos resultados, é possível concluir que animais mantidos na altura de 25 cm apresentaram ganhos ótimos por área e por animais enquanto que a suplementação com suplemento enriquecido com gordura protegida apresentaram os melhor resultados. Contudo, o uso da suplementação deve ser atenciosamente analisado, haja vista que esta estratégia torna o sistema mais sensível economicamente.

Palavras-chave: altura do dossel, metano e suplementação.

MARANDU GRASS MANAGEMENT AND SUPPLEMENTATION ON PERFORMANCE AND METHANE MITIGATION OF NELLORE BULLS

Abstract

Canopy height and supplementation is the mainly strategies to increase animal production or area gain in system on pasture. However, some reporter diverge about the correct management in tropical forages, furthermore, the effect of supplementation with soybean differently available in rumen in pasture still inconclusively. By this sense, two trials were conducted to evaluate the effect of supplementation for young Nellore bulls grazing *Brachiaria brizantha* cv. Marandu with different sward height on herbage characteristics, dry matter intake, digestibility, animal performance and gain per area during the rainy season. Hundred and eight animal young Nellore bulls were used. Treatments corresponded to three different canopy height (15, 25 and 35 cm) when years where analyzed and the combined with sward height and supplementation with mineral salt, supplement with soybean grain or supplement with protect fat. The level of supplementation was 0.5% of BW. Pastures were fertilizer according to local recommendations following soil analysis plus 135 and 90 kg of nitrogen by urea during first and second year, respectively. Increase the canopy height increased herbage mass whilst decreased stocking rate. There was no difference in hand-plucked herbage CP, NDF and IVDMD concentration among treatments. Supplemented animals had higher DM intake and digestibility values of all nutrients except protein when compared to the control treatment. However, the supplements did not affect the production of CH_4 expressed as g CH_4/day. The increase in liveweight gain using either supplement caused a decrease in the production CH4/ kg product and g CH4/kg DMI. Considering that relation between animal performance and stocking rate, marandu pastures should be not be grazed below 20-cm stubble or above 30-cm for greater productivity. By other hand, supplementation with lipid of low ruminal degradation can increase performance with higher effect of protect fat source and reduce CH_4 emission (g CH_4/kg liveweight gain) largely by the increase in intake rather than a reduction in methane production per se. This provides a practical way in which methane emissions per unit of product might be decreased for grazing cattle.

Keyword: canopy height, methane, supplementation.

1. INTRODUCTION

Ruminants nowadays play an important role in human nutrition. The first ruminants evolved around 50 million years ago, and their domestication started around 10,000 B.C. with the husbandry of goats for meat production (ZEDER AND HESSE, 2000). Their purpose was initially for meat production, but later, ruminants were also used for milk production, draught power, transportation, currency and in religious rituals (CLUTTON-BROCK, 1999). The global population of domesticated ruminants is currently estimated at 3.6 billion, nearly 50 times as large as the population of wild ruminants (HACKMANN AND SPAIN, 2010).

The global livestock sector is rapidly changing in response to globalization and growing demand for animal source food, driven by population growth and increasing wealth in much of the developing world. Moreover, there is a growing appreciation that the livestock sector needs to operate in a carbon-constrained economy, resulting in increasing competition for land and water resources, and growing pressure for the sector to be managed cleanly, safely and balanced (FAO, 2011).

Extensive cattle ranching is the predominant grassland-based activity in Brazil, and has been responsible for over 90% of the stocking rate (Animal Unit Equivalent, AU) since 1995. Most of the cattle herd (83.5%) is related to beef production (IBGE, 2006). This being so, it seems reasonable to assume that the environmental challenges to pastoral farming systems in Brazil will mostly concern the beef sector.

Ruminants exist symbiotically with microorganism in the rumen which ferment both starchy, pectin and fibrous feed to volatile fatty acids and form microbial protein. Archeal methanogens typically account for < 1% of the microbial rumen population, but play an important role in fermentation by preventing H_2 accumulation through the reduction of CO_2 to CH_4. Although CH_4 production appears necessary for efficient fermentation, it represents an energetic loss up to 12% of the gross energy intake of the host (JHONSON AND JHONSON, 1994).

By this sense, pasture management decisions, such as grazing systems or adjust of stocking rate in function of forage allowance, can improve the quality of pasture available to grazing herd. Any reduction in the CH_4 emission from grazed pasture arising from an improvement in quality should be exploited because will reflect in improvement of productivity besides decrease GHG emission.

Methane emission can be decreased by supplementing the diet with certain additives and ingredients. Adding concentrate to the diet might reduce methane emission by lowering ruminal fiber fermentability, and to a lesser degree, through hydrogenation (JOHNSON AND JOHNSON, 1995). Reducing methane production can be of direct economic benefit it coincides with greater energy-use efficiency of the feed by the animal.

2. LITERATURE REVIEW

2.1. Grazing managements

The structure of the sward has 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).

Intake and digestibility are the two factors that determine total nutrient supply or metabolizable energy intake by an animal, whether housed or grazing. Allden and

Whittaker (1970) showed that intake was limited by the herbage mass on offer and the allowance or pasture dry matter (DM) offered/head/day. Both these parameters (herbage mass and allowance) have an underlying outcome, that of utilization, i.e. the amount or proportion of the herbage on offer that is removed during a grazing event, usually expressed on a daily basis.

In practical terms, intake and animal performance (liveweight gain) follow a curvilinear asymptotic function in response to allowance, which is used in managing animals at pasture either to achieve maximum performance or maximum herbage utilization (ALLDEN AND WHITTAKER, 1970; MILLIGAN ET AL., 1987).

Thus rules for the minimum amount of herbage mass on offer to maximize intake and the need to offer animals at least four times more feed than they could consume on a daily basis to maximize intake (CARVALHO ET AL., 2013) evolved and became part of management plans. Poppi et al., (1987) identified nutritional constraints as limits to intake when allowance was high (at plateau for the response curve) and harvesting constraints as limits to intake for the ascending part of the response curve of intake to allowance. This pattern appears to hold for temperate and tropical grasses and legumes.

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 and structure on forage intake of grazing cattle has been reported by several other authors (DA SILVA; CARVALHO, 2005; CASAGRANDE et al., 2011; PAULA et al., 2012).

Where sward are more horizontally dispersed, animals will select from horizons or patches of highest accessible leaf density and that may be at the base of the sward (L´HUILLIER et al., 1986). Across the landscape of rangeland pastures, similar principles apply for site selection and plant species, both of which may vary markedly in the horizontal plane. The drive to select leaf mass or accessibility declines (STOBBS, 1973; CHACON AND STOBBS, 1976). Animals try to accommodate for a smaller bite size by increasing grazing time available to an animal on a daily basis and the consequence of a reduced bite size is frequently a reduction in intake. The extra effort of harvesting leaf may in fact lead to a reduced grazing time. Stems have a negative influence in that they are not selected and may be regarded as partial aversion, often (but not always) lower in nutrient content, and may pose a barrier to the animals ability to harvest leaf (STOBBS, 1973; BENVENUTTI et al., 2006, 2008, 2009).

By this sense, the correct pasture management should develop strategies for increase leaf allowance and maximize total nutrient intake. Therefore, the adjustment of the stocking rate to maintain optimum grazing pressure is essential. Thus, studies on canopy height resulted in a better understanding of the response of forage crops and animals to pasture intensity.

2.2. Supplementation

Energy supplementation can be used during summer or wet season, where the forage has higher CP content compared with winter or dry season. In well managed tropical grass, the level of CP (CORREA, 2006; COSTA, 2007; PACHECO

JR., 2009) is enough to supply animal requirement for a growing beef cattle, being the energy the most limiting nutrient.

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 might reduce intake of grazed forage.

Supplementation of energy may alter energy requirements of grazing ruminants by altering grazing behavior or by influencing efficiency of nutrient use (CATON AND DHUYVETTER, 1996). Data of Sarker and Holmes (1974) and Adams (1985) indicate that supplementing concentrates will decrease grazing time. If grazing time is decreased, energy demands from work associated with grazing should also decrease. Additionally, Adams (1985) indicated that degree of change in grazing time associated with supplementation depended on time of supplementation. Other work (DELCURTO ET AL., 1990; MINSON, 1990) indicated little change in grazing time in response to supplementation. These authors did not report data on efficiency of energy use. In general, energy supplementation reduces grazed forage intake in ruminants (HORN AND MCCOLLUM, 1987; PATERSON et al., 1994). In some cases, especially with sheep, forage intake is stimulated by lower levels of supplemental grain. Providing supplemental energy as readily digestible fiber usually has a less negative effect on forage intake than starch-based supplements and can result in increases in total intake. While providing supplemental energy in the form of grain increases efficiency of energy use (NRC, 1984), the impact of readily digestible fiber sources on these efficiencies is less well-defined.

Energy supplementation is responsible for increasing volatile fatty acids, such a 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 mechanism of propionate in the liver and its action on the hungry control is scarce.

Energy supplementation from grains can reduce ruminal pH levels (SANSON et al., 1990). However, current data clearly suggest that responses are not consistent and that at times ruminal pH is not greatly affected by grain supplementation, especially at moderate to low levels of supplementation. Use of readily degradable fiber sources as energy feeds has yielded production responses similar to those to grain supplements (ANDERSON et al., 1988). Their impact on ruminal pH is less defined and seems to be related to level of supplementation. In the case in which energy supplements, regardless of source, begin to substitute for large amounts of basal forage, reductions in ruminal pH and, hence changes in ruminal environment, often occur. At lower levels of supplementation, responses are much less predictable. It seems that some of the data indicate that decreases in forage

The use of supplements for grazing animals is a practice that can be used in pasture management strategy to increase the carrying capacity and animal performance. This requires sound knowledge on the subject, in order to achieve maximum technical and economic efficiency.

2.3. GHG emission from ruminant

As the human population grows, humanity will become increasingly reliant on the ability of ruminant livestock to convert the fibrous biomass, unsuitable for human consumption, into meat and milk, protein of high quality (McALLISTER et al., 2013). However, livestock are increasingly scrutinized for their GHG emissions, as the two primary GHGs produced by livestock production systems, nitrous oxide (N_2O) and CH_4, have global warming potentials 298 times and 25 times greater than CO_2, respectively (IPCC, 2007).

The CH_4 enteric production by ruminants is higher when they are feed with low nutritional value, composed of high fiber with low digestibility (KURIHARA et al., 1999; BEAUCHEMIN et al., 2008; ARCHIMEDE et al., 2011). In lactating dairy cows fed a pasture based diet, emissions can be as high as 330 g of CH_4/day (GRAINGER et al., 2007) or ~460 L of CH_4 gas. Degradation of lignocelluloses in ruminants by microbes in the reticulo-rumen results in formation of microbial fermentation products which are absorbed and used by the animal as energetic precursors and subsequent growth and productivity.

However, in anaerobic environments, organic material is decomposed by bacteria through the process of fermentation, where organic material is broken down to, among others, VFA and carbon dioxide. Hydrogen, released during the production of VFA, accumulates in the fermentation system. In an aerobic environment, oxygen would be the terminal electron acceptor and oxygen would be reduced to H_2O, using the excess hydrogen in the process. The lack of oxygen in anaerobic systems necessitates the use of other terminal electron acceptors to remove hydrogen from the fermentation system. A number of compounds can be used for this purpose (ferric iron, sulfate, nitrate, manganese and carbon dioxide). However, these compounds, except for carbon dioxide, are only present in low concentrations in most fermentation systems. The compounds that are normally present in low concentrations (ferric iron, sulfate, nitrate and manganese) are rapidly reduced and exhausted and carbon dioxide functions as the main terminal electron acceptor for the remaining excess hydrogen. Carbon dioxide is reduced to methane in the fermentation system (Figure 1), and the methane in gaseous form subsequently dissipates from the system.

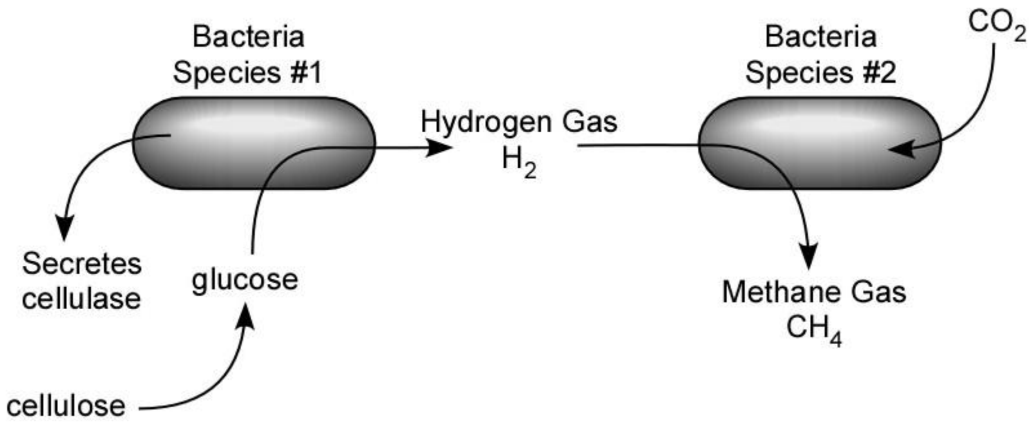

Figure 1. Illustration simplified of the ruminal methanogenesis.

Source: www.courses.bio.indiana.edu

Ruminants rely on microbial fermentation to a larger extent than other species and methane emissions from ruminant species expressed per kilogram of body weight are relatively high for this reason (Table 1). During the production of acetate (equation 2) and butyrate (equation 4), hydrogen is produced, but the production of propionate (equation 3) results in the net uptake of hydrogen. A higher proportion of propionate in the VFA-profile therefore results in reduced methane production (ELLIS et al., 2008) and this property can be utilized in the manipulation of methane production. However, the production of acetate and butyrate always exceeds propionogenesis, resulting in a net surplus of hydrogen in the rumen.

$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$ (equation 1)

$C_6H_{12}O_6 + 2H_2O \longrightarrow 2C_2H_3O_2 + 2CO_2 + 4H_2 + 2H$ (equation 2)

$C_6H_{12}O_6 + 2H_2 \longrightarrow 2C_3H_5O_2 + 2H + 2H_2O$ (equation 3)

$C_6H_{12}O_6 \longrightarrow C_4H_7O_2 + H + 2H_2 + 2CO_2$ (equation 4)

Reduce the methanogenesis, require create another metabolic pathway (WEIMER, 1998), reducing the supply of the H_2 to methanogenic microbial population with the propionic acid increase production, or reducing the protozoa population, because these microorganisms produces H_2 (MORGAVI et al., 2010).

According to Russel and Wilson (1996), the diet changes could modify the ruminal microorganism population. The supplementation with soluble carbohydrate are unfavorable to cellulolytic population, and favorable to amylolytic population, propionic acid producers. Therefore, the supplementation with concentrates will be favorable to the production of propionic acid than acetic acid, reducing methanogenesis.

There is a high correlation between production efficiency and methane (CH_4) emission reduction, which is a rumen fermentation co-product, highly associated with environmental impact. From a nutritional standpoint, well-balanced diets with low fiber and high digestibility decrease energy losses and increase animal performance (BOLAND et al., 2013), and these sense, the same result can be observed in well managed tropical grasses pastures (REIS et al. 2014, VALENTE et al., 2014).

2.4. Manipulating the rumen environment

Manipulating ruminal methanogenesis has been the focus of many CH_4 mitigation strategies yet, very few have been successful at persistently reducing CH_4 production. Enteric CH_4 production by methanogens is reduced if the flow of H_2 shifted towards alternative electron acceptors. However, as the reduction of many of the alternative acceptors is less thermodynamically favorable than the reduction of CO_2 to CH_4, fermentation inadvertently reverts back to the production of CH_4 (McALLISTER et al., 2013).

Given the relatively simple range of substrates used by methanogens, it is not clear why so many methanogen types exist in the rumen. It is likely that ruminal methanogen diversity reflects the niches that can support methane generating archaeal lifestyles, such as the planktonic, particulate associated and ectosymbiotic associations within the reticulo-rumen, as well as fluctuating substrate concentrations, especially H_2, which depend on animal diets and plant fibre degradation rates. For example, the hydrogenotrophic Methanobrevibacter, which convert H_2 + CO_2 (or formate) to CH_4, are represented by at least three species in the rumen (JANSSEN and KIRS, 2008). Presumably these Methanobrevibacter species experience a range of H_2 concentrations in the rumen, and they may be differently adapted to use H_2 at specific concentrations. Alternatively, these species may rely on a supply of H_2 directly from H_2 producing organisms, and may be specially adapted to grow in close association with those organisms. Furthermore, Methanosarcina have been isolated from ruminants fed molasses (ROWE et al., 1979; VICINI et al., 1987), alfalfa, corn and soybean meal (PATTERSON and HESPELL, 1979) or from pastured ruminants (JARVIS et al., 2000), but are not thought to constitute substantive populations under most ruminal conditions (ATTWOOD et al., 2011).

The potential of dietary strategies to handle the rumen environment and reduce methane emission by ruminants has been acknowledged such the better way (TAMMINGA et al., 2007, BEAUCHEMIN et al., 2008, MARTIN et al., 2010, ECKARD et al., 2011, BERCHIELLI et al., 2013). Dietary strategies mainly revolve around one of the following principles (JOBLIN, 1999; MARTIN et al., 2010):

- Direct inhibition of methanogenesis
- Lowering of the production of hydrogen during fermentation
- Providing alternative pathways for use of hydrogen in the rumen

Many ingredients or plant extracts (lipids, tannins, saponins, ionophoros, essential oil, etc) have been screened for their potential to directly inhibit methanogenesis. The results of these screening are very promising, but verification of their efficacy remains necessary.

2.5. Lipid supplementation

The supplementation of lipids is considered the most effective way of depressing ruminal methanogenesis (MacHMULLEr et al., 2000; MARTIN et al., 2010), at least in the short term. Although reductions of up to 40% of CH_4 emissions can be achieved with high levels of lipid supplementation (MacHMULLER and KREUZER, 1999; JORDAN et al., 2006), reductions of 10–25% are more likely, as the level of lipids in ruminant diets must be limited to 6 – 8% dry matter (DM) to avoid negative impacts on feed intake and carbohydrate digestion (BEAUCHEMIN et al., 2008).

Dietary fat addition has been shown to reduce methane production by ruminants in many studies (JORDAN et al., 2006; MacHMULLER, 2006; MARTIN et al., 2008; FIORENTINI et al., 2014; VALENTE et al., 2014). Increase lipid content in the feed is thought to decrease methanogenesis through inhibition of protozoa, increased production of propionic acid, and by “biohydrogenation of unsaturated fatty acid”. Other way of lipids might decrease methane emission is associated mainly with a decrease the proportion of fiber fermentable, or in the diet, which in itself would decrease methane emissions (MacHMULLER et al., 2000; BEAUCHEMIN et al., 2009; MARTIN et al., 2010). Unsaturated fatty acids may be used as hydrogen acceptors as an alternative to the reduction of carbon dioxide. Also, fatty acids are thought to inhibit methanogens directly through binding to the cell membrane and interrupting membrane transport.

A meta-analysis of methane output with lipid supplementation in lactating dairy cows found a 2.2% decrease in methane per 1% of supplemented lipid in the diet (EUGÈNE et al., 2008). In cattle and sheep, Beauchemin et al., (2008) reported an association of 5.6% methane reduction per percentage unit of lipid added to the diet. There are many factors that may account for varying effects of lipids on methane abatement, such as the ruminant species, experimental diet, and the type of lipids used.

Fiorentini et al., (2014) in stud with different source of the fat in beef cattle added palm oil (PA), linseed oil (LO), protected fat (PF) and whole soybean grain (WSG) in diet containing 42g of additional lipid/kg of DM achieved which diets with LO, PO and WSG caused an average decrease of 30% in the emission of enteric CH_4 (g/kg DMI) compared with diets without fat and protect fat. The relationship between CH_4 emission (g/d) and DMI is positive but characterized by high variability between animals (KURIHARA et al., 1999). According to Lassey et al. (1997), approximately 87% of the variation in CH_4 emissions is attributed to differences between animals and only 13% is due to differences in DMI. Therefore, the intrinsic characteristics of the animals are a major cause of variation in the amount of CH_4. According Lassey et al. (2002), these variations can occur in zebu, taurine, and crossbred animals and may be associated with distinct characteristics of the animals, such as the capacity of feed selection, ruminal volume, retention time of feed in the rumen, and association factors that lead to greater or lesser capacity for fiber digestion.

Beauchemin and McGinn (2006) added canola oil (4.6% DM) to a high-forage diet and effectively suppressed CH_4 emissions, as a percentage of GEI, by 21% in dairy and beef cattle. However, emissions relative to digestible energy (DE) intake were only 6% lower than the control, indicating the negative impacts of feeding high dietary lipid concentrations. As such, the expression of CH_4 in terms of DE may be beneficial, as it eliminates the confounding effects of intake and digestibility on emissions (McALLISTER et al., 2013).

Factors such as the level of supplementation, the source of fat and associated FA profile, and the form in which the fat is administered (i.e. refined oil or oilseeds) can result in highly variable responses. The negative effects of feeding oil on intake and digestion could be attenuated by releasing lipids more slowly into the rumen, perhaps by supplementing lipids in the form of whole or moderately processed oilseeds rather than as free oil (DHIMAN et al., 2000). By this sense, low ruminal degradation lipid sources can be absorb on the intestine without reduce forage degradability from the animals, increasing the nutrients and forage intake and, reduce the CH_4 enteric emission.

No matter what the lipid form used for supplementation, it is important to consider the ruminant species and the diet being examined, as methane reduction can vary depending on the feed components present. Further, lipid inclusion can affect palatability, intake, animal performance, beef and milk components, all of which can have implication for practical on-farm use (ODONGO et al., 2007; JORDAN et al., 2006).

The persistence of the effects of lipids on CH_4 production has yet to be confirmed and remains an important avenue of research. Ivan et al. (2004) and Grainger et al. (2008; 2010) reported reductions in CH_4 emissions can be maintained for up to 3 months with the addition of lipids. However, the potential for rumen microflora to adapt to shifts in diet composition may result in lipid supplementation having a transient impact on CH_4 emissions (McALLISTER et al., 2013).

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INCREASE CANOPY HEIGHT ENHANCE PERFORMANCE OF YOUNG NELORE BULL GRAZING MARANDU PASTURE

Abstract: The objective of experiment was to describe the effect of three different canopy heights and relate them to herbage intake, grazing behavior and animal performance. Pastures were sampled for herbage mass, leaf and stem bulk and chemical composition. Thirty six young Nellore bulls (each year) grazed three different heights [Brachiaria *brizantha* (Hochst ex A. Rich) Stapf cv. Marandu] in completely randomized design split in six paddocks (with two paddocks each treatment and six animals) four periods and two years were analyzed. The animals were weighed at the beginning and end of each experimental period. The herbage evaluations were recorded each 28 days. Herbage intake rate was analyzed using a two marker technique. Morphological components and animal variables differ ($P>0.05$) between the years. The herbage intake rate and behavior grazing differed between treatments ($P<0,001$). Young bulls grazing high pasture had greatest animal performance (0.8 kg d^{-1}) and herbage intake (2.12% BW), however shown the lower stocking rate (3.5 UA ha^{-1}). The lower canopy height (15 cm) occurred with the highest stock rate (6.2 UA ha^{-1}) and DM digestibility (658 g/kg), although had lower herbage mass (4.8 Mg DM ha^{-1}) and average daily gain (0.4 kg d^{-1}). The highest productivity, that is, animal performance (0.6 kg d^{-1}) by stock rate (4.6 UA ha^{-1}) occurred with a canopy height of approximately 25 cm.

Keyword: animal performance; *Brachiaria brizantha*; canopy height;

1. INTRODUCTION

The gross value of beef production in Brazil is approximately US$58.5 billion, and as such, makes an important contribution to Brazilian´s economy. Beef cattle production in Brazil is largely based on extensive grazing of tropical pastures with Brachiaria pasture with approximately 100 million ha (MAPA, 2013).

In Brazil, seasonal rainfall distribution results in rapid growth of pasture during the spring/summer period, which then declines in quality as the season advance and the plants mature and senesce. The pattern of cattle growth from these pasture systems closely follows the seasonal pattern of pasture growth with weight loss commonly occurring during the dry season followed by wet season weight gain (REIS et al., 2009).

Grazing intensity has been recognized as the most important grazing management factor affecting the availability of pasture nutrients to grazing livestock (NEWMAN et al. 2002). Likewise, it is the primary determinant of animal production, whether on a per animal or per unit land area basis. More intensively grazed tropical grass pasture has less biomass but greater herbage bulk density than taller pasture (SOLLENBERGER and BURNS, 2001), often resulting in less opportunity for livestock to prehend leaf and negatively affecting performance (CHACON and STOBBS, 1976).

One of the main factors for the intensification of grazing-based animal production systems is the correct management of pasture. Therefore, the adjustment of the stocking rate to maintain optimum forage allowance is essential (CASAGRANDE et al., 2011). In this sense, the objective of this research was to assess the qualitative, quantitative and structural variables of forage canopies and liveweight gain of young Nellore bulls kept Marandu pastures under a continuous stocking system with different heights of grazing during the wet season.

2. MATERIAL AND METHODS

The experiment was conducted in the forage experimental sector of the Animal Science Department at FCAV/UNESP, Jaboticabal, São Paulo, Brazil (21º15'22" S 48º18'58" W, alt. 595 m) over two consecutive wet seasons(January until April), 2011 and 2012. The protocol used on this experiment was in accordance with Colégio Brasileiro de Experimentação Animal – COBEA (Brazilian College of Animal Experimentation) guidelines and was approved by the UNESP Ethics, Bioethics, and Animal Welfare Committee. The experimental area consisted of six experimental paddocks with an area of 0.7, 1.0 or 1.3 ha in two replication with *Brachiaria brizantha* (Hochst ex A. Rich) Stapf cv Marandu pasture established in 2001 on an Oxisol. According to Köppen classification the climate is subtropical type Awa, with rainy summers and dry winters.

Pastures were fertilized according to local recommendations following soil analysis. The first year 200 kg ha^{-1} of 04:14:08 ($N:P_2O_5:K_2O$) were applied in December 29, 2010, subsequent fertilizer applications were implemented on 18 January 2011; 18 February 2011 and 23 March 2011, with nitrogen (urea) at a rate of 45 kg ha^{-1}. In the second year, pastures were fertilized on 1 December 2011, through the application of 250 kg ha^{-1} of 10:10:10 ($N:P_2O_5:K_2O$). Subsequent fertilizer applications were implemented on 27 December 2011 and 25 February 2012, with nitrogen at a rate of 45 kg ha^{-1}.

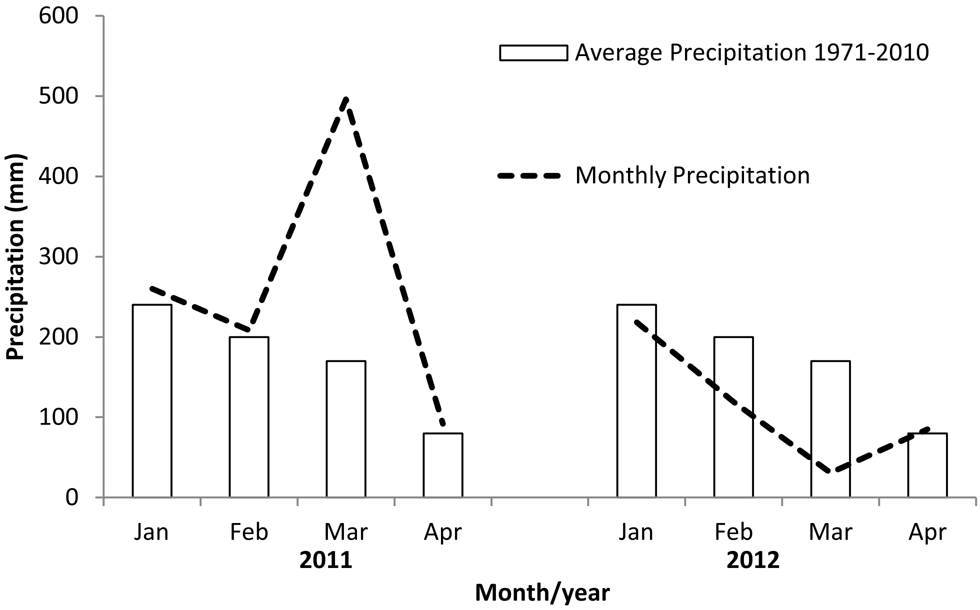

Figure 1: Average monthly precipitation from 1971 to 2010 and during the experimental period (2011 and 2012) at the Animal Science Department at FCAV/UNESP, Jaboticabal, Sao Paulo, Brazil

The treatments were three canopy heights 15, 25 and 35 cm, with two replicates (paddock) per forage height. Six young Nellore bulls were used per paddock, totaling 36 animals per wet season (12 per treatment) with an average initial liveweight of 220 kg (first wet season) and 243 kg (second rainy season). The grazing method utilized was continuous stocking with a variable stocking rate to maintain canopy heights, in addition 15 put-and-take animals with the same age and weight were used to control canopy height. Forage height was measured once a week at 100 random points in each paddock and stocking rate adjusted to maintain target canopy heights.

Pastures were sampled for herbage mass (HM) just prior to initiation of grazing and every 28 d during the grazing period in each experimental paddock. The HM was measured by two methods. The indirect measure was the settling height of a 0.25-m^2 aluminum disk, and the direct measure involved hand clipping all herbage from 2 cm above soil level within a 0.5 x 0.5 m area. Every 28 d, five samples by each method were taken from each of the six experimental paddocks. Sites were chosen that represented the average disk height of the pastures. At each site, the disk settling height was measured and the forage clipped. Clipped forage was dried for 72 h and weighed.

Five sites (0.25 m^2) were clipped at 2 cm above soil level in each paddock and the samples were hand-separated into leaf (live and dead blade plus sheath) and stem (including inflorescence, if present) components and dried at 60°C until constant weight to determine leaf:stem ratio.

Hand-plucked samples were used to estimate nutritive value of the grazed portion of the canopy. One sample was taken per pasture every 28d. The samples were plucked to simulate the steers grazing as closely as possible. The grazing behavior of the animals was observed during sampling after prior familiarization. The samples were dried at 60^{o}C in a forced-air oven to constant weight, ground in a Wiley mill to pass a 1mm stainless steel screen, and taken to the laboratory for analyses.

Intake and digestibility estimates were evaluated during seven consecutive days in the intermediate phase of the experiment. To evaluate the individual intake and apparent digestibility of each animal a two marker technique was used as described by Ferreira et al., (2009), using LIPE® and indigestible NDF (iNDF).

The product LIPE® (lignin that was isolated, purified, and enriched from *Eucalyptus grandis*, UFMG, Minas Gerais) was used to estimate faecal excretion. During six days, a capsule of 500 mg of this marker was dosed orally in six animals (3 animals/ paddock) from each treatment, the first three days were for marker equilibration and faeces were sampled at once by day over the final three days (SANTOS et al., 2012).

Faecal samples were dried (60°C for 72 h), ground (1 mm), and subsequently bulked over the three day collection period. Approximately 10 g of composite faeces from each animal were sent to the Federal University of Minas Gerais (Universidade Federal de Minas Gerais) for faecal production estimation, as described by Saliba (2005).

To estimate the individual forage intake, iNDF was used as an internal marker. For the quantification of iNDF in the samples of faeces and forage, these materials were placed in Ankom filter bags (F57; Ankom Tech. Corp., Fairport, NY) and incubated in the rumen of a fistulated steer for 264 h (CASALI et al., 2008). The remaining material from the incubation was washed in water and then subjected to extraction with neutral detergent, with the residue considered to be iNDF. The individual forage intake was estimated by subtracting the excretion of this marker relative to the concentrated from the total excretion of iNDF and dividing this difference by the concentration of iNDF in the forage.

Samples of concentrated, forage, and faeces were analysed for dry matter (DM), organic matter (OM) and crude protein (CP) (VAN SOEST and ROBERTSON, 1985). The CP was estimated using the Dumas combustion method, on the Leco®, model FP- 528 (Leco Corporation, Michigan, USA). Ether extract was analyzed by the Soxhlet extraction with petroleum ether. The concentration of NDF corrected for ash and protein was determined by the technique adapted from Mertens (2002) and acid detergent fiber ADF was analyzed using Ankom Tech. Corp., Fairport, NY.

Cattle were weighed at initiation (07 January 2011 and 27 January 2012) of the experiment and every 28 d thereafter. Weights were taken at 08:00 h following a 16-h feeding and water fasting. Average daily gain was calculated from the initial and final weight (04/29/2011 and 05/16/2012), while the intermediate weights were used to calculate stock rate.

The evaluation of herbage mass, pasture structure, nutritive value of the forage and performance, intake, and stock rate of the animals were used a completely randomized design with three treatments, two replicates (paddocks), four periods (months) and two years using a mixed model of the MIXED procedure of SAS software, version 9.2 (SAS, 2008). Year was considered fixed because of the potential for carry-over effects of treatments from Years 1 and 2. Replicate and its interactions were considered random effects. Periods were analyzed as repeated measures. Treatments were considered different when $P < 0.10$. Interactions not discussed were not significant ($P > 0.10$). The means reported are least squares means adjusted by the polynomial contrast were used to compare linear and quadratic effect of height. The means reported are least square means and were separated by Fisher´s protected least significant difference (LSD) at $P < 0.10$.

3. RESULTS AND DISCUSSION

3.1. Herbage Responses

Contrasting rainfall between years was reported. During the experimental period rainfall was 1056 and 453 mm for 2011 and 2012, respectively, whilst N fertilizer was 28kg of N greater in the first year.

Table 1: Effects of canopy height on herbage responses of *Brachiaria brizantha* cv. Marandu pasture. Data are mean across replicates (two replicates for canopy height), 4 mo, and 2 yr.

Response variable	Canopy height			Polynomial contrast		SE	Year		SE
	15	25	35	L	Q		1	2	
Herbage responses (Mg ha^{-1})									
Herbage mass	4.8	8.4	10	<0.01	0.05	0.5	8.4^{a*}	7.2^{b}	0.5
Leaf mass	1.4	2.4	2.8	<0.01	0.37	1.7	2.2^{a}	2.1^{b}	0.1
Steam mass	1.4	2.9	3.3	<0.01	0.01	1.4	2.8^{a}	2.1^{b}	0.1
Dead material	1.9	2.9	3.8	<0.01	0.94	2.2	3.3^{a}	2.9^{b}	0.1
Chemical composition (g kg^{-1})									
Crude protein	146	146	136	0.12	0.38	0.45	156^{a}	130^{b}	0.36
Neutron detergent fiber	590	594	603	0.24	0.83	0.78	611^{a}	580^{b}	0.62
IVDMO	609	628	604	0.81	0.30	1.64	620^{a}	607^{b}	1.34

IVDMO: in vitro organic matter digestibility; *Means followed by the same lowercase letter within rows are not different ($P>0.10$)

Herbage mass increased linearly from 4.8 to 10 Mg ha^{-1} as stocking rate decreased from low to high canopy height. The effect of stocking rate on HM has been reported in the literature, and a decrease in HM with greater stocking rate is a consequence of increased total forage intake of a greater number of animals. According Sollemberger et a., (2012) the choice of grazing intensity is more important than any other single grazing management decision (JONES and JONES, 1997; SOLLENBERGER and NEWMAN, 2007) because of its prominent role in determining forage plant growth and persistence (CHACON and STOBBS, 1976), forage mass and allowance (BURNS et al., 2002; HERNÁNDEZ GARAY et al., 2004) animal performance (HUMPHREYS, 1991; NEWMAN et al., 2002) size of nutrients pools (THOMAS, 1992; DUBEUX et al., 2006) and profitability of the grazing operation. The greater HM reported in first year was a consequence of the highest rainfall during the study (Figure 1), furthermore the additional N fertilization (28 kg N) likely increased herbage mass. According to Mislevy et al., (2001) and Ezenwa et al., (2006), higher temperatures may result in rapid grass growth. Management practices and climatic conditions affect plant structure, especially bulk density and leaf (FAGUNDES et al., 1999) responsible for variation in the forage height. In addition, the maintenance of a constant forage stubble height during the growing season is challenging to implement in commercial livestock enterprises owing to the variation in growing conditions and limited opportunities for constantly stocking rate.

Table 2: Animal responses of young Nellore bulls grazing *Brachiaria brizantha* cv. Marandu pasture at three canopy height. Data are mean across replicates (two replicates for canopy height) and 2 yr.

Response variable	Canopy height			Polynomial contrast		SE	Year		SE
	15	25	35	L	Q		1	2	
Dry matter intake (kg d^{-1})	5.42	6.40	6.99	<0.01	0.23	1.05	6.07	5.94	0.13
Dry matter intake (% BW)	1.78	2.02	2.12	0.08	0.40	0.22	2.01	1.95	0.32
Grazing time (hours)	7.22	6.62	6.12	0.04	0.34	0.20	6.65	6.89	0.19
Initial body weight (kg)	249	252	256	0.40	0.96	5.5	261^{a*}	243^{b}	4.4
Final body weight (kg)	295	323	345	0.01	0.67	5.8	334^{a}	308^{b}	4.8
Average daily gain (kg d^{-1})	0.4	0.6	0.8	0.02	0.45	0.03	0.6^{a}	0.5^{b}	0.02
Stocking rate (UA ha^{-1})	6.2	4.6	3.5	<0.01	0.13	0.10	5.0^{a}	4.5^{b}	0.08
Gain per hectare (kg ha^{-1} d^{-1})	4.1	4.4	3.9	0.99	0.20	0.19	4.5^{a}	3.8^{b}	0.16

UA: 450 kg liveweight; *Means followed by the same lowercase letter within rows are not different (P>0.10)

There was a linear increase in LM as canopy height increased (Table 1). However, the proportion of leaf decreased 30, 29 and 28% as canopy height increased, 15, 25 and 35 cm, respectively. Pastures grazed at low stocking rate (high canopy height) had decreased % leaf, likely because excess HM may result in self-shading, accumulation of nonphotosynthetic residue, especially on the young basal tillers (ADJEI et al., 1980; PARSONS et al., 1988; HERNANDEZ GARAY et al., 2000). In addition, young bulls in this experiment likely selected for leaves in the diet, and this may have decreased their proportion in the canopy (BURNS and SOLLENBERGER, 2001).

There was no difference in hand-plucked herbage CP, NDF and IVDMD concentration among treatments (Table 1). In previous studies with warm-season grasses that increasing stocking height increased forage nutritive value (INYANG et al., 2010; HERNANDEZ GARAY et al., 2004), mainly because of shorter intervals between grazing events at high stocking rate. According Sollenberger et al., (2012) in a recent review found that nutritive value response to increasing grazing intensity was not as consistent as the forage quantity response, yet nearly all studies (40 of 41; 98%) reported either no effect (13 of 41; 32%) or positive effect (27 of 41%; 66%) on nutritive value. Only one (2%) reported a negative effect (ACKERMAN et al., 2001). In three of the 13 studies showing no effect, the author cited the relatively narrow range of stocking rate imposed as a reason for lack of response (VALENCIA et al., 2001; ARTHINGTON et al., 2007; SCAGLIA et al., 2008).

However, difference in nutritive value was reported between years, likely because the rainfall in first year was greater than second year. The increase in forage nutritive value with greater grazing intensity may seem counterintuitive because there is less forage mass and grazing occurs at lower strata in the canopy. Nutritive value generally decreases from top to bottom of a canopy, particularly for C4 grasses (FISHER et al., 1991; HOLDERBAUM et al., 1992). However, when canopies are grazed intensively over an extended period of time the leaf proportion of the forage mass is greater and age of regrowth is younger because of shorter intervals between animal visits to individual patches (ROTH et al., 1990; PEDREIRA et al., 1999; NEWMAN et al., 2002a, 2002b; DUBEUX et al., 2006).

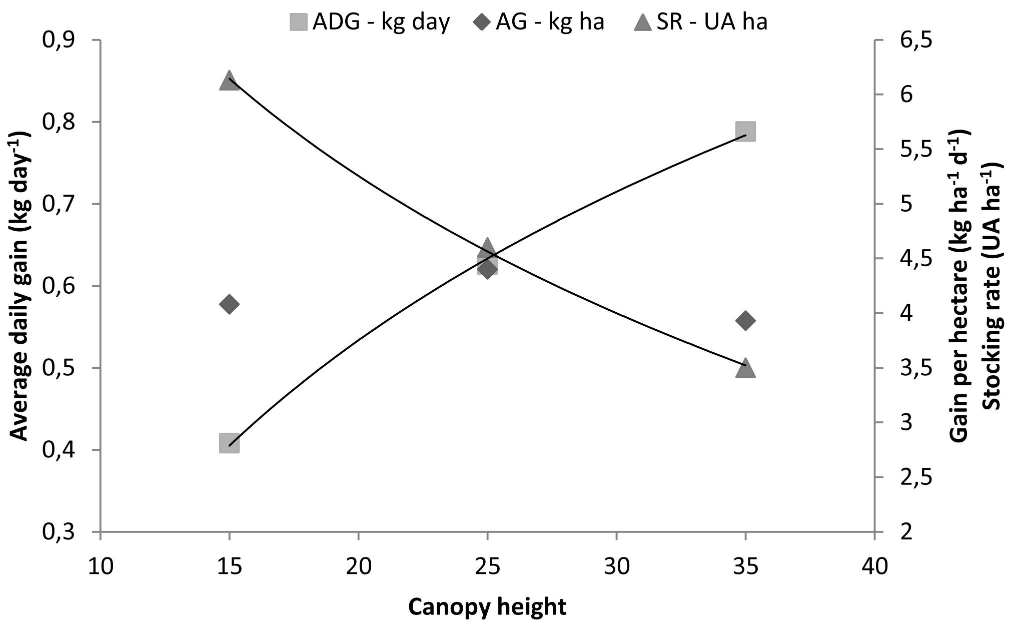

Figure 2: Relationship among average daily gain (ADG), gain per hectare (AG) and stocking rate (SR) of young Nellore bulls grazing *Brachiaria brizantha* cv. marandu pasture at three canopy height.

3.2. Animal Responses

The main goal of pasture management is to maximize intake of nutrients by grazing ruminants. On this sense, the ability of a grazing animal to harvest herbage appears the most important factor limiting intake when intake is responding steadily to increases in bite mass (POPPI et al. 1987). Variation in herbage bulk density may contribute independently to bite mass (HODGSON et al. 1994). Although variation in sward height generates a wider range in intake bite mass, intake tends to increase asymptotically responding to sward height. This is influenced by sward structure and grazing behavior (diet selection and grazing time), characteristics that are strongly influenced by grazing management practices. In this context, lower canopy heights increased grazing time (7.22 h) while a taller canopy decreased grazing time (6.12 h). However, increases in grazing time did not reflect increases on dry matter intake, therefore, dry matter intake was 4.72, 6.40 and 6.99 kg/day for canopy heights of 15, 25, and 35 cm, respectively. In this sense, grazing intensity (defined as stocking rate or canopy height) is regarded as the primary determinant of animal performance and that live weight gain was 400, 625, and 775 g/d for 15, 25, and 35 cm, respectively.

Animal performance was more related to intake (Table 1) than to the nutritive value of the herbage, since there was no difference in chemical composition of the ingested herbage. Under continuous stocking of tropical pasture, Chacon and Stobbs (1976) demonstrated that nutrients available to the animal can mainly be altered by the grazing intensity of the sward. More intensively, grazed tropical grass pastures have less biomass but greater herbage bulk density than taller pastures, often resulting in less opportunity for livestock to prehend leaf and negatively affecting performance. Lenient grazing increases the opportunity for selection and facilitates prehension, but stocking rates are lower. Under grazing can also be harmful; with the build-up of material of low nutritive value, shading reduces photosynthetic activity and the forage production may be reduced and decrease animal productivity per unit of area.

In tropical grasses, as sward height increases, leaf lamina length increases linearly, while the increase of leaf mass per unit of leaf lamina length is quadratic (CASTRO, 2002; SILVA, 2004). This indicates that the ascending section of the functional response curve is not strictly a function of increasing bite volume, a progressive decline can be expected in the rate of mass acquisition per unit of bite volume from tall swards, when leaf mass per unit of leaf lamina length reaches a plateau; and for tropical species, it is important to describe leaf lamina structure (DA SILVA and CARVALHO, 2005).

Therefore, both intensity and timing of defoliation is of the greatest importance in pasture productivity. In this regard, the primary aim of animal production is the balance between production per animal and per unit of area (kg live weight/ ha). Thus, pasture management in 25 cm showed greater results between production per animal and per area. Similar managements have direct and indirect impacts on sward control, structure and animal performance that need to be known in order to allow the correct planning and decision making process on a farm scale.

4. SUMMARY AND CONCLUSIONS

Stocking rate affected forage and animal responses on *Brachiaria brizantha* cv. Marandu pasture. Increasing canopy height enhanced herbage mass and animal performance however decreased stocking rate. Even at the greatest stocking rate in pasture managed in 15 cm achieved the lower productivity whilst greater gain per hectares was shower by animals grazing 25 cm of pasture. It is likely that further increase in stocking rate would result in a quadratic gain per hectare response. The decision of the proper canopy height must be related to the climatic conditions and fertilizer application and grazing method. Considering that relation between animal performance and stocking rate, marandu pastures should be not be grazed below 20-cm stubble or above 30-cm for greater productivity.

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METHANE EMISSION FROM YOUNG NELLORE BULLS GRAZING BRACHIARIA AND SUPPLEMENTED WITH SOYBEAN OIL DIFFERENTIALLY AVAILABLE IN THE RUMEN

Abstract: The emission of greenhouse gases (GHG) resulting from ruminant production systems has contributed to global warming. A variety of research has been reported to decrease CH_4 emission without affecting animal performance. However, there is no information about the effect of addition of lipids with differing availability in the rumen in supplement to cattle grazing tropical pasture. The objective of this experiment was to investigate whether supplementation with whole soybean grain (WSG) or protected soybean oil (PSO) can increase liveweight gain and decrease CH_4 emission of young Nellore bulls grazing *Brachiaria brizantha* pasture. This experiment was carried out in the Department of Animal Science, São Paulo State University (UNESP) located in Jaboticabal, São Paulo, Brazil from January to May 2012. A total of 36 bulls of approximately 18 months of age and initial live weight of 243±24.2 kg were distributed in a completely randomized design consisting of 3 treatments and 2 replicates (paddocks). The dietary treatments were as follows: mineral salt (C), whole soybean grain (WSG), and protected soybean oil (PSO). Supplements were offered and consumed at 5g/kg of BW/animal daily (DM basic). Ruminal parameters were evaluated in 3 Nellore steers fitted with ruminal cannulas and allocated to a 3 x 3 Latin square design. Within each experimental period, NH_3-N, pH, and molar % of volatile fatty acids were recorded at 0, 3, 6, 9, 12, and 24 h. Pastures evaluated were collected each 28 days to analyze herbage mass and canopy structure. No statistical differences were detected between the paddocks concerning the variables of herbage mass (mean 6232 ± 2680.9kg DM/ha) and herbage mass of leaf blades (mean 1797 ± 790.5 kg DM/ha). No differences in chemical composition (DM basis) were observed in the pastures. Both lipid sources ($P<0.05$) increased intake and liveweight gain. Supplemented animals had higher DM intake and digestibility values of all nutrients except protein when compared to the control treatment. However, the supplements did not affect the production of CH_4 expressed as g CH_4/day. The increase in liveweight gain using either supplement caused a decrease in the production CH4/ kg product and g CH4/kg DMI. There were effects of the different lipid availabilities in the rumen ($P<0.05$) on NH_3-N, acetate, propionate, and the acetate: propionate ratio and no significant difference in pH ($P=0.185$). The supplementation with lipid of low ruminal degradation can increase performance and reduce CH_4 emission (g CH_4/kg liveweight gain).

Key words: methane, tropical pasture, soybean source

1. INTRODUCTION

The emission of greenhouse gas (GHG) resulting from ruminant production systems has contributed to global warming, accounting for approximately 10–12% of global estimated GHG emission (Lima et al., 2001 and 2006; Smith et al., 2007). Alternatives to improve the efficiency of feed conversion may benefit the production system and the environment. The proportion of concentrate within the diet has been reported to be negatively correlated with methane emission (Yan et al., 2000). Lovett et al. (2005) demonstrated that increased concentrate use at pasture reduced enteric methane per kilogram of animal product. Nevertheless, improvement in pasture management by offering animals forage with better nutritive value may be an efficient strategy to reduce methane missions (Crosson et al., 2011).

Research on reduction of CH_4 emissions has shown that the manipulation of diet, especially the use of lipid supplements, is a promising strategy (Eugène et al., 2011). However, previous research suggested that the inhibitory effect of fat on CH_4 emission depends on the concentration, lipid source, composition of fatty acids, and nutrients that compose the diet (Beauchemin et al., 2008; Machmuller, 2006; McAllister et al., 1996). Fiorentini et al., (2013) and Beauchemin et al., (2008a) found no change in digestibility of feedlot rations with the addition of lipids from soybean or protected soybean but there is no information for grazing cattle in tropical conditions. Lipids can have a direct effect on rumen bacteria, e.g., *B. fibrisolvens*, *R. albus*, and *R. flavefaciens* and reduce intake (Nagaraja et al., 1997), and protected lipids may avoid this effect. The effect on methane production is not known.

In this sense, the objective of this paper was to determine the effect of adding supplements with whole soybean grain (WSG) or protected soybean oil (PSO) with different availabilities in the rumen on the CH_4 emission, intake, and liveweight gain from young Nellore bulls grazing *Brachiaria brizantha* cv. Marandu.

2. MATERIAL AND METHODS

The experiment was conducted from January to May 2012 in the Department of Animal Science, São Paulo State University (UNESP) located in Jaboticabal, São Paulo, Brazil. All experimental protocols used were in accordance with the COBEA (Brazilian College of Animal Welfare and Ethics) FCAV – UNESP – Jaboticabal campus.

Thirty-six Nellore bulls were used with initial average weight of 243 ± 24.2 kg and approximately 18 months of age. Bulls were grazed on *Brachiaria brizantha*

(Hochst ex A. Rich) Stapf cv. Marandu pasture, established in 2001 on Red Oxisol. The experimental area was divided into six paddocks of 1.0 ha each. Each treatment was replicated with six animals in each paddock. According to Köppen classification the climate is subtropical type Awa, with rainy summers and dry winters. Climatic records during the experiment are shown in Table 1.

2.1. Treatments and feeding management

The treatments were: 1) control basal pasture *Brachiaria brizantha* with *ad libitum* salt minerals (BellNutri, Bellman, Mirassol, SP, Brazil); 2) control pasture supplemented with 500g/kg DM with WSG in the supplement; and 3) control pasture supplemented with 111 g/kg of protected soybean oil (Lactoplus® – Dalquim group, Itajaí, Santa Catarina, Brazil) in the supplement. This equated to approximately 50g fat/kg of dry matter intake. The animals received 5 g/kg BW of supplement daily.

The composition of the diets and lipid sources is reported in Table 2. Pasture was maintained at a sward height of 25 cm using continuous grazing but variable stocking rate. The balance between gains per animal and per hectare is obtained with swards grazed at 25 cm (Casagrande et al., 2011; Reis et al., 2013). To adjust the pasture height, ten regulator animals were used. After determining the average forage height, measured weekly with a sward stick graduated in centimeters, the regulator animals were removed or put in the paddocks (Sollenberger and Cherney, 1995).

Pastures were fertilized on December 1, through the application of 250 kg ha^{-1} of 10:10:10 ($N:P_2O_5:K_2O$). Subsequent fertilizer applications were implemented on 12/27/2011 and 02/25/2012, with nitrogen at a rate of 45 kg ha^{-1}.

Table 1: Average monthly precipitation during the experimental period (January to May 2012) at the Animal Science Department at FCAV/UNESP, Jaboticabal, Sao Paulo, Brazil

Months	P	T min	T max	T average
			°C	
January	219	28.9	19.0	22.8
February	119	31.8	19.7	25.0
March	31	31.5	19.0	24.2
April	85	30.2	18.2	23.2
May	73	26.2	14.4	19.4

P: average monthly rainfall.

Pastures were sampled for herbage mass (HM) just prior to initiation of grazing and every 28 d during the grazing period in each experimental paddock. 2HM was measured by two methods. The indirect measure was the settling height of a 0.25-m^2 aluminum disk, and the direct measure involved hand clipping all herbage from 2 cm above soil level within a 0.25 m^2 quadrant. Every 28 d, five samples by each method were taken from each of the six experimental paddocks. Sites were chosen that represented the average disk height of the pastures. At each site, the disk settling height was measured and the forage clipped. Clipped forage was dried for 72 h and weighed. At sampling every 28 d, 80 sites per experimental unit for disk measures were chosen by walking a fixed number of steps between each drop of the disk to ensure that all sections of the pasture were represented. The average disk height of the 80 sites was entered into the equation to predict actual HM. The HM from the clipped sample and the corresponding disk height were used to develop a regression equation, which was later used to estimate HM. Five sites (0.25 m^2) were clipped at 2 cm above soil level in each pasture and the samples were hand-separated into leaf (live and dead blade plus sheath) and stem (including inflorescence, if present) components and dried at 60 °C until constant weight to determine leaf:stem ratio.

Hand-plucked samples were used to estimate nutritive value of the grazed portion of the canopy. One sample was taken per pasture paddock every 28 d. The samples were plucked simulating grazing by steers as closely as possible. Grazing behavior of the animals was observed during sample collection after prior familiarization. The samples were dried at 60 ^{o}C in a forced-air oven to constant weight, ground in a Wiley mill to pass a 1mm stainless steel screen, and taken to the laboratory for analyses.

2.2. Chemical analysis, intake, performance, and methane production

Intake and digestibility estimates were evaluated during 10 consecutive days in the intermediate phase (between days 120 and 130) of the experiment. To evaluate the individual intake and apparent digestibility of each animal, a three marker technique was used as described by Ferreira et al. (2009), using LIPE®, titanium dioxide, and indigestible neutral detergent fiber (iNDF).

LIPE® (lignin that was isolated, purified, and enriched from *Eucalyptus grandis*, UFMG, Minas Gerais, Brazil) was used to estimate fecal excretion. During six days, a capsule of 500 mg of this marker was dosed orally in 6 animals per treatment (3 animals/paddock), with the first 3 days for marker equilibration, and feces was sampled once a day over the final 3 days, always in the morning (Santos et al., 2011).

Table 2: Supplement ingredients and chemical composition (% DM basis) of experimental diets.

Ingredients	Diets		
	Control	Whole soybean grain	Protected soybean oil
Cornmeal	0.0	46.0	45.0
Soybean meal	0.0	0.0	40.0
Soybean grain	0.0	50.0	0.0
Protected lipid	0.0	0.0	11.0
Mineral supplement[1]	100	4.0	4.0
Chemical composition			
Dry matter[2]	0.0	89.1	88.3
Organic matter[3]	0.0	94.2	94.2
Ash[3]	0.0	6.7	7.1
Crude protein[3]	0.0	23	22
Ether extract[3]	0.0	12	12
TDN[4]	0.0	81.7	80.2

[1/]Commercial nucleus Bellman, BellNutri (Ca Min./Max. 139/155 g/kg; P. 80g; Mg. 10g; E. 40g; Na. 130g; Co. 1350mg; Mn. 1040mg; Zn. 5000mg; I. 100mg; Co. 80mg; Se. 26mg; F. 1333mg); [2/]Percentage; [3/]DM percentage; [4/]Total digestible nutrients according to NRC 2001.

Fecal samples were dried (60 °C for 72 h), ground (1 mm), and subsequently bulked over the 3-day collection period for each animal. Approximately 10g of composite feces from each animal were sent to the Federal University of Minas Gerais (Universidade Federal de Minas Gerais) for lignin analysis and estimation of fecal production, as described by Saliba (2005).

Titanium dioxide was used to estimate the individual intake of lipid, provided in the supplement from the supplement trough (Ferreira et al., 2009). During 10 days, 10g of the marker was provided per bull, mixed into the concentrate provided to the group. This schedule involved a total of 11 days of application, with 6 days for the marker stabilization and 5 days for the fecal collection, which was performed once a day at alternate hours (first day: morning, second day: afternoon).

Fecal samples were dried (60 °C for 72 h), ground (1 mm), and samples bulked per bull over the 5-day collection period. The samples were analyzed for titanium dioxide, using the technique described by Myers et al. (2004).

To estimate the individual forage intake, indigestibility neutral detergent fiber (iNDF) was used as an internal marker. For the quantification of iNDF in the samples of feces, concentrate, and forage, these materials were placed in Ankom filter bags (F57; Ankom Tech. Corp., Fairport, NY) and incubated in the rumen of a fistulated steer for 264 h (Casali et al., 2008). The remaining material from the incubation was washed in water and then subjected to extraction with neutral detergent, with the residue considered to be iNDF. The individual forage intake was estimated by subtracting the excretion of this marker relative to the concentrated from the total excretion of iNDF and dividing this difference by the concentration of iNDF in the forage.

Samples of supplement, forage, and feces were analyzed for dry matter (DM), organic matter (OM), and crude protein (CP) (Van Soest & Robertson, 1985). CP was estimated using the Dumas combustion method, on the Leco®, model FP-528 (Leco Corporation, Michigan, USA). Ether extract was analyzed by Soxhlet extraction with petroleum ether. The concentration of neutral detergent fiber corrected for ash and protein (NDF) was determined by a technique adapted from Mertens (2002); supplements were analyzed with the addition of thermostable alpha-amylase enzyme

(F57; Ankom Tech. Corp., Fairport, NY). Lignin was determined after cellulose extraction with 70% sulfuric acid (Van Soest & Wine, 1967).

Cattle were weighed at initiation (01/27/2012) of the experiment and every 28 d thereafter. Weights were taken at 0800 h following a 16-h feed and water fast. Average daily gain was calculated from the initial and final weights (05/16/2012), while the intermediate weights were used to adjust the supplementation levels.

The daily CH_4 emission was measured for 6 days between 04/20/2012 and 04/26/2012 using the sulfur hexafluoride (SF_6) tracer technique (Johnson & Johnson, 2004). Randomly,six animals from each treatment (3 animals/paddock) were selected for the purpose of measuring the emission of CH_4. The release rate (RR) of the gas from a permeation tube is known before its insertion into the rumen. The permeation tubes were maintained in a water bath at 39 °C and weighed for 6 weeks in the laboratory. The average RR was similar among the treatments (RR 2.04 ± 0.5 mg SF6/d, mean ± SD). Brass permeation tubes filled with SF6 and with known release rates were administered orally to each of the 20 animals 72 h before methane sampling to allow the tracer gas to equilibrate in the rumen. Animals were fitted with gas collection halters connected to pre-evacuated polyvinyl chloride canisters designed to fill halfway in 24 h. Sampling started at 0700 h daily, when the animals were removed from the paddock, and was conducted at the management center (stockyard) to facilitate sampling.

The collection canisters were located above each animal to reduce the risk of equipment damage and were connected to the halter by tubing inside airline-flexible coil tubing. The pressure of the canister was measured after 24 h of collection, and if the final pressure was beyond the expected range, then the halter was preventively replaced. If the final pressure was above the range, then the halter was most likely blocked or disconnected. If the final pressure was below the expected range, then there could be a leak in the system. In either situation, the halter was replaced with a new halter with an average absorption rate within the stipulated range (to fill halfway over a 24-h period).

After sampling (approximately 30 min), each animal was taken to the original paddock. The pressure readings were recorded, and the canisters were pressurized using pure N_2. The ambient air samples in the experimental area were collected daily in two different positions beyond the experimental area to determine the background concentrations of methane and SF6. These values were subtracted from the animals' values to calculate the net output in the expired breath. The concentrations of CH_4 and SF_6 in the collection tubes were measured at Embrapa Meio Ambiente (Jaguariúna, SP, Brazil) using a Hewlett Packard model 6890 gas chromatograph (Agilent, San Jose, CA, USA), as described by Johnson and Johnson (1994).

2.3. Metabolic Analysis

Three Nellore steers (420 ± 30 kg BW), fitted with ruminal fistulas, were allocated to a 3 x 3 Latin square design containing 3 periods of 21 d each. This potentially may not have accounted for potential carryover treatment effects across periods and cannot be balanced for residual effects. The same paddocks and treatments were those used for the measurement of animal performance. Within each experimental period, adaptive time was evaluated from d 1 to 20. Treatments were measured on d 21, to assess rumen fermentation parameters. Rumen fluid samples (around 80 mL) were collected manually, both before supplying the diet (time zero) and 3, 6, 9, 12, and 24 h after feeding. Immediately after collection, the pH of rumen fluid was determined using a digital potentiometer (ORION 710A, Boston, MA). A 2-mL aliquot of collected fluid was placed into a plastic bottle and frozen at -20 °C for subsequent volatile fatty acid (VFA) analysis according to a method adapted from Erwin et al. (1961). A 40-mL aliquot of rumen fluid was used for ammonia N (NH_3-N) analysis following the methodology adapted by Fenner (1965).

2.4. Statistical Analyses

The evaluation of herbage mass, pasture structure, and nutritive value of the forage used a completely randomized design with three treatments, two replicates (paddocks) and periods using a mixed model of the MIXED procedure of SAS software, version 9.2 (SAS, 2008). The main effects were analyzed by Tukey test at 5% probability. The interactions between the factors studied were divided using the SLICE option of SAS, with periods being the dividing factor.

The completely randomized design was used to evaluate the intake, digestibility, methane production, and animal performance with two replicates (paddocks), three treatments. Statistical calculations were performed using the GLM procedure of SPSS version 19.0, and the means were compared by Tukey test at 5% probability. The following statistical model was used in the calculation of variables:

$$Yijkl\ Tj = \hat{A}\mu + Al\ (i) + eijkl$$

Where *Yijkl* is the dependent variable; *Â*µ is the average of observations; *Tj* is the *j* – effect of treatment or diet; *Al* is the effect of column or animal; *eijkl* is the random error, assumed normally and independently distributed error.

All data were analyzed using steers as the experimental unit for metabolic analyses. Treatment effects on ruminal parameters were analyzed using the PROC MIXED procedure of SAS and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement contained the effects of treatment, in addition to period as independent variable. Data were analyzed using steers as the random variable. Results are reported as least square means and were separated using PDIFF. Significance was set at $P \le 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected.

3. RESULTS

No statistical differences were detected between the paddocks concerning the variables of herbage mass (mean 6232 ± 2680.9kg DM/ha), herbage mass of leaf blades (mean 1797 ± 790.5 kg DM/ha) or ratio of leaf/stem DM (mean 0.91 ± 0.15). No differences in chemical composition (DM basis) were observed for organic matter (mean 91.0 ± 0.50 %), crude protein (mean 13.6 ± 1.39%), ether extract (mean 1.8 ± 12.13%), neutral detergent fiber (mean 60.4 ± 1.14%), acid detergent fiber (mean 26.0 ± 0.81%), or DMD (mean 60.8 ± 0.93%) of the pasture. The average stocking rate between the paddocks was 2025 kg live weight/ha. The cut-off value employed was $P < 0.10$.

Both lipid sources ($P < 0.05$) increased intake and liveweight gain (Table 3). Supplemented animals had higher DMI, expressed as kg/day and % BW, than control animals but different responses for forage intake were obtained depending on whether this variable was calculated using % BW or kg DM/day. Control animals consumed a larger amount of forage (2.13% BW) compared to animals receiving supplements with WSG (1.91% BW) or PSO (1.92% BW). However when intake was calculated using kg DM/day, the total DMI was higher for animals supplemented with WSG (6.71 kg) and PSO (6.84 kg) when compared with the control treatment (6.26 kg).

Differences were also found in the digestibility of nutrients, where the strategy of using physically (WSG) or chemically protected lipid sources did not depress fiber digestibility. Supplemented animals showed higher digestibility values of all nutrients except protein when compared to the control treatment. It is worth noticing that ether extract digestibility was 40% higher in supplemented animals than the controls (Table 3).

Supplementation with different soybean oil availabilities in the rumen did not affect the production of CH_4 expressed as g CH_4/day (Table 4). The increase in liveweight gain (Table 3) using either supplement caused a decrease in the production CH4/ kg product and g CH4/kg DMI (Table 4). There were no significant interactions between both lipid availabilities in the rumen and evaluation time for the variables of pH, NH_3-N, acetate, propionate, and the acetate: propionate ratio (Table 5). However, there were effects of the different lipid availabilities in the rumen ($P<0.05$) on NH_3-N, acetate, propionate, and the acetate: propionate ratio and no significant difference in pH ($P=0.185$).

4. DISCUSSION

This experiment examined the effect of two lipid availabilities in the rumen on the CH_4 emission of grass-fed young Nellore bulls. Bulls supplemented with either lipid source had the highest liveweight gain and lowest CH_4/kg liveweight gain or DM intake. Previously differences in methane emission in response to lipids have been observed but there were variable effects on intake and liveweight gain (Cooke et al., 2011).

Table 3: Average daily weight gain (ADG), total dry matter (DM) intake, and apparent digestibility by young Nellore bulls grazing a *Brachiaria brizantha* cv. Marandu pasture receiving different lipid source supplements.

Variable	Supplements				
	C	WSG	PSO	SEM	P
ADG (g)	622^{b}	930^{a}	1020^{a}	0.171	<0.001
Intake - % BW/d					
	1.9^{b}	2.4^{a}	2.4^{a}	0.337	0.004
Intake - kg/day					
DM	6.2^{b}	8.3^{a}	8.5^{a}	0.31	<0.001
OM	5.0^{b}	7.6^{a}	7.7^{a}	0.32	<0.001
CP	0.9^{b}	1.2^{a}	1.2^{a}	0.26	<0.001
EE	0.1^{b}	0.3^{a}	0.3^{a}	2.36	<0.001
NDF	3.6^{b}	4.0^{a}	4.1^{a}	0.14	<0.001
Apparent Digestibility - g/kg					
DM	687.8^{b}	754.2^{a}	739.0^{a}	0.90	<0.001
OM	702.5^{b}	768.6^{a}	758.2^{a}	0.70	<0.001
EE	505.4^{b}	824.4^{a}	817.8^{a}	0.76	<0.001
CP	773.5	786.1	776.0	1.01	0.02
NDF	667.1^{b}	681.0^{a}	680.4^{a}	0.70	<0.001

Control (C), whole soybean grain (WSG), protected soybean oil (PSO), average daily gain (ADG), total dry matter intake (DM), forage (F), supplement (S), organic matter (OMI), crude protein (CP), ether extract (EE), and nitrogen neutral fiber (NDF).

^{a,b}Means within a row with differing superscripts differ (Tukey's test, $P < 0.001$).

Saturated fatty acids, as found in greater concentration in grain, or rumen-protected forms may be particularly useful because these have minimal effects on rumen microbial activity but on the other hand have little effect on methane emission (Palmquist & Jenkins, 1980). Sources of fat with different availabilities in the rumen may influence responses through effects on ruminal fermentation and nutrient digestion.

Methane emission, expressed as g CH_4/kg DM intake, was approximately 7.2g lower (8% lower) in the supplemented than in control animals, however this was not statistically different (P=0.557). The increase in DM and OM intake in response to supplementation increased animal performance resulting in lower methane production expressed in g CH_4/kg liveweight gain. Mertens (1994) suggested that 60 to 90% of animal performance may be attributed to the DMI. In this regard, both lipid sources with different availabilities in the rumen, as used here, increased total DM intake with no effect on digestibility and so an increase in liveweight gain was expected.

In reviewing 17 studies with beef, sheep, and dairy cattle, Beauchemin et al., (2008) concluded that for every 1% (DMI basis) increase in fat in the diet, CH_4 (g/kg DMI) was reduced by 5.6%. Assuming that most forages have some fat content, and that DMI may be suppressed at fat intakes above 6 to 7%, CH_4 abatements of 10–25% are possible from the addition of dietary oils to the diet of ruminants. There are four possible mechanisms by which lipid supplementation reduces CH_4: reducing fiber digestion (mainly by action of saturated acids); lowering DMI (if total dietary fat exceeds 6–7%); suppression of rumen protozoa; and to a limited extent through biohydrogenation (Beauchemin et al., 2008; Johnson and Johnson, 1995; McGinn et al., 2004).

Table 4: Methane emission of young Nellore bulls grazing *Brachiaria brizantha* cv. Marandu pasture and receiving different lipid supplements.

Variable	Treatments				
	C	WSG	PSO	SEM	P
g CH_4/Day	89.5	96.1	97.3	13.16	0.557
g CH_4/kg liveweight gain	143.9^a	94.2^b	104.6^b	16.86	<0.001
g CH_4/kg DMI	14.3^a	11.6_b	11.6_b	0.311	<0.001

Control (C), Whole soybean grain (WSG), Protected soybean oil (PSO), Grams of methane per day (g CH_4/Day), grams of methane per kg of liveweight gain (g CH_4/kg liveweight gain), and grams of methane per kg of dry matter intake.
[a,b]Means within a row with differing superscripts differ (Tukey's test, $P < 0.001$).

Table 5: The molar % of volatile fatty acids and the ammonia and pH levels in the rumen of young Nellore bulls grazing *Brachiaria brizantha* cv. Marandu and receiving different lipid supplements.

	Diets			SEM	P
	C	WSG	PSO		
Acetic acid	76.6^a	73.1^b	74.2^b	1.696	<0.001
Propionic acid	15.1^b	17.6^a	15.5^b	1.639	0.003
Acetic:Propionic	5.07^a	4.36^b	4.84^{ab}	0.588	0.019
NH3 – N, mg/dL	14.5^b	16.4^a	15.3^{ab}	0.420	0.052
pH	6.6	6.4	6.5	0.107	0.185

Control (C); whole soybean grain (WSG); protected soybean oil (PSO).
[a,b]Means within a row with differing superscripts differ (Tukey's test, $P < 0.001$).

However, the effectiveness of adding lipids to the diet to reduce CH_4 emission depends on many factors including level of supplementation and fat source (BEAUCHEMIN et al., 2008). The direct action of the lipid within the rumen is thought to occur by these mechanisms but protected lipid sources would not have this effect and so the action must be on intake and liveweight gain rather than directly on methane emission (Wanapat et al., 2011). Not all lipid from whole soybean would be physically protected but the current results suggest the effect is due more to intake and subsequent liveweight gain effects.

According to Czerkawski et al. (1966), the use of non protected fat above 3–4% in the diet can reduce rumen microbial activity and cellulose digestibility. However, in this study, supplementation did not decrease the digestibility of NDF. This may have been due to the large proportion of lipid which was protected in this study and so minimal effect on fiber digestion.

An important outcome of the higher growth rates is that it is possible for the animal to reach slaughter weight at a younger age. This means a reduction in methane emission over the life of the animal (Smith et al., 2007).

In this sense, use of supplementation for animals in pasture is a important technology, however the supplementation response was shown to be dependent on the forage allowance, nutritive value, and sward structure (Reis et al., 2009). The forage nutritive value in this study was high for a tropical grass, where the frequent averages are 90 g/kg CP and 501 g/kg DM digestibility in Brazil in the wet season (Figueiredo et al., 2008; Porto et al., 2009; Zervoudakis et al., 2008). In pasture, there is a strong relationship between CP, NDF, digestibility, leaf mass, intake, and animal performance. Marandu grass pasture was maintained at 25 cm height during the wet season and comprised CP (135 g/kg), NDF (603 g/kg), and DM digestibility (608 g/kg), which resulted in average liveweight gain of 0.65 kg/day in this study. However, supplementation resulted in intake substitution (Table 3) which agrees with Moore (1980).

For maintenance and growth, the animal tissues require a source of amino acids for protein production, and a source of energy. The forage evaluated in the study had 190 g CP/kg digestible OM, which is within the range suggested by Poppi and McLennan (1995) and Poppi et al. (1997) to optimize the rumen microbial protein synthesis. Supplementation with lipid sources in this experiment provided an additional gain of 308 to 398 g/animal/day for WSG and PSO respectively, seemingly in direct response to the higher nutrient intake (Table 3).

An increased proportion of starch in the diet changes the concentration of ruminal volatile fatty acid (VFAs) in such a way that less acetate and more propionate is formed, and the supply of hydrogen for methanogenesis is limited. Also, the pH decreases as the proportion of propionate increases, which reduces methanogenic activity (Walichnowski & Lawrence, 1982). Higher concentrate feeding has reduced methane output by reducing the protozoal population (Van Soest, 1994). However, supplementing with WSG or PSO did not increase the concentration of propionate enough to decrease CH_4 emission.

5. CONCLUSION

This research showed that cattle grazing tropical forage and supplemented with lipid of low ruminal degradation can increase performance and reduce CH_4 emission (g CH_4/kg liveweight gain) largely by the increase in intake rather than a reduction in methane production per se. This provides a practical way in which methane emissions per unit of product might be decreased for grazing cattle.

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RELATIONSHIP BETWEEN CANOPY HEIGHT AND SUPPLEMENTATION IN ANIMAL PERFORMANCE

Abstract: The objective of the present experiment was to describe the effect of three different canopy heights (15, 25 and 35 cm) and relate with tree supplementation strategic (supplement with whole soybean grain, by pass fat or control). Were available them forage characteristics, herbage and supplement intake and animal performance. Pastures were sampled for herbage mass, leaf and stem bulk and chemical composition. Hundred eight young Nellore bulls grazed three different heights [Brachiaria *brizantha* (Hochst ex A. Rich) Stapf cv. Marandu] in factorial design split in eighteen paddocks (with two paddocks each treatment and six animals) were analyzed. The animals were weighed at the beginning and end of each experimental period. Herbage evaluations were recorded each 28 days. There was difference in the pastures variables only between heights without effect on supplement. Pasture maintained on 15 cm showed high SR (5.93 UA/ha) whilst this value decreased when increased the height, 25 cm (4.54 UA/ha) and 35 cm (3.43 UA/ha). The chemical composition did not differ between canopy height and supplement. The averages were 131 g/kg of crude protein; 579 g/kg of neutral detergent fiber; 261 g/kg of acid detergent fiber and 567 g/kg of *in vitro* dry matter digestibility. The strategic supplementation increased digestibility in the 15 and 25 cm of canopy height. Average daily gain varied with canopy height being that swards managed at tall height resulted in greater ADG (0.88 kg/d). Animals receiving PF showed the highest value (0.95 kg/d) while animals with mineral salt achieved lower performance (0.57 kg/d).

Key word: animal performance, pasture management, supplementation

1. INTRODUCTION

Brazil has historically been one of the biggest producers of bovine meat and in the last five years, the largest exporter of cattle meat in the world. According to USDA (2011) one in each five pounds of commercialized cattle meat is from Brazil. However, an extensive cattle ranching is the predominant grassland-based activity in Brazil, and has been responsible for over 90% of the stocking rate since 1995. Most of the cattle herd (83.5%) is related to beef production (IBGE, 2006). In this regards, Brazil has about 100 million hectares of cultivated pastures and 60% of this pasture is of *Brachiaria brizantha* cv. Marandu.

The production cycle conducted in Brazil is exclusively on grazing systems with only mineral supplementation which leads older animals at market, mainly because cattle gain body weight during the rainy season (summer) and loses body weight during the dry season (winter). Intensify grazing systems for maximum livestock production per unit of land area is necessary, in this sense, improve pasture management is the better way. According Newman et al., (2002) grazing intensity has been recognized as the most important grazing management factor affecting the availability of pasture nutrients to grazing livestock

Brazil is a tropical country and the seasonal rainfall distribution results in rapid growth of pasture during the spring/summer period. However, there is a nutritional unbalance in tropical pasture during rainy season, which not result on the optimal performance. Therefore, there is an unused potential growth of approximately

200g/animal/d, which can be achieved by using supplement (Poppi and McLennan, 1995). In this sense, use of supplements for grazing animals is a practice that can be used in pasture management strategy to increase the stock rate and animal performance. Nevertheless, this requires sound knowledge on the subject, in order to achieve maximum technical and economic efficiency.

The objective of this research was to assess the effect of canopy height and supplementation on pasture qualitative, quantitative and structural variables of forage canopies and liveweight gain of young Nellore bulls maintained Marandu pastures under a continuous stocking system during the wet season.

2. MATERIAL AND METHODS

The experiment was conducted in the forage experimental sector of the Animal Science Department at FCAV/UNESP, Jaboticabal, São Paulo, Brazil (21º15'22" S 48º18'58" W, alt. 595 m) during wet seasons in 2012 (Table 1). The protocol used on this experiment was in accordance with Colégio Brasileiro de Experimentação Animal – COBEA (Brazilian College of Animal Experimentation) guidelines and was approved by the UNESP Ethics, Bioethics, and Animal Welfare Committee.

Pastures were fertilized according to local recommendations following soil analysis. The pastures were fertilized on 1 December 2011, through the application of 250 kg ha^{-1} of 10:10:10 ($N:P_2O_5:K_2O$). Subsequent fertilizer applications were implemented on 27 December 2011 and 25 February 2012, with nitrogen at a rate of 45 kg ha^{-1}.

2.1. Treatments and experimental design

The experimental area consisted of eighteen experimental paddocks, being six for each area (0.7, 1.0 or 1.3 ha) with *Brachiaria brizantha* (Hochst ex A. Rich) Stapf cv Marandu pasture established in 2001 on an Oxisol. According to Köppen classification the climate is subtropical type Aw, with rainy summers and dry winters.

Table 1: Average monthly precipitation during the experimental period (January to May 2012) at the Animal Science Department at FCAV/UNESP, Jaboticabal, Sao Paulo, Brazil

Months	P	DR	T min	T max	T average
				°C	
January	219	22	28.9	19.0	22.8
February	119	11	31.8	19.7	25.0
March	31	8	31.5	19.0	24.2
April	85	7	30.2	18.2	23.2
May	73	7	26.2	14.4	19.4

P: average monthly rainfall; DR: day of rainfall.

The effect of three forage heights was studied, combined with the offer of three dietary supplements, totaling nine treatments with two replications (paddocks). The canopy height were 15, 25 and 35 cm, and the supplements were composed of mineral salt (BellNutri, Bellman, Mirassol, SP, Brazil) - control treatment; supplement with 500 g/kg DM with whole soybean grain (WSG) and supplement with 111 g/kg of by-pass fat (BF) (Lactoplus® - Dalquim group, Itajai, Santa Catarina, Brazil). Animals were supplemented with 0.5% of BW daily and the mineral salt was provided ad libitum and replenished weekly. The composition of the diets and lipid source is reported in table 2.

Six young Nellore bulls were used per paddock, totaling 108 animals with an initial body weight 243 ± 27.3 kg and 10 months age. The grazing method utilized was continuous stocking with a variable stocking rate to maintain canopy heights. In addition 27 put-and-take animals with the same age and weight were used to control canopy height. Forage height was measured once a week at 100 random points in each paddock and stocking rate adjusted to maintain target canopy heights. At the end of each period, the number of grazing days of all of the animals in a plot were summed and divided by the number of days in the period to obtain the stocking rate of each plot.

Combinations of canopy height and supplementation were applied in the plots according to size: plots with a smaller area (0.7 ha) had a forage height of 15 cm, those of 1.0 ha had a forage height of 25 cm, and the largest area (1.3 ha) had a forage height of 35 cm. Thereby, same number of test animals per paddock were obtained.

Table 2: Supplement composition (% DM basis) of ingredients and chemical composition of the diet.

Ingredients	Diets		
	Control	Whole soybean grain	Protected soybean oil
Cornmeal	0.0	46.0	45.0
Soybean meal	0.0	0.0	40.0
Soybean grain	0.0	50.0	0.0
Protected lipid	0.0	0.0	11.0
Mineral supplement[1]	100	4.0	4.0
Chemical composition			
Dry matter[2]	0.0	89.1	88.3
Organic matter[3]	0.0	94.2	94.2
Ash[3]	0.0	6.7	7.1
Crude protein[3]	0.0	23	22
Ether extract[3]	0.0	12	12
TDN[4]	0.0	81.7	80.2

[1/]Commercial nucleus Bellman, BellNutri (Ca Min./Max. 139/155 g/kg; P. 80g; Mg. 10g; E. 40g; Na. 130g; Co. 1350mg; Mn. 1040mg; Zn. 5000mg; I. 100mg; Co. 80mg; Se. 26mg; F. 1333mg); [2/]Percentage; [3/]DM percentage; [4/]Total digestible nutrients according to NRC 2001.

2.2. Sward measurements

2.2.1. Herbage mass determination and sampling

Pastures were sampled for herbage mass (HM) just prior to initiation of grazing and every 28 d during the grazing period in each experimental paddock. The HM was measured by two methods. The indirect measure was the settling height of a 0.25-m^2 disk, and the direct measure involved hand clipping all herbage from soil level within a 0.25-m^2 area. Every 28 d, five samples by each method were taken from each paddock. Sites were chosen that represented the average disk height of the pastures. At each site, the disk settling height was measured and the forage clipped. Clipped forage was dried for 72 h and weighed.

Five sites (0.25 m^2) were clipped at soil level in each paddock and the samples were hand-separated into leaf (live and dead blade plus sheath) and stem (including inflorescence, if present) and dead material components and dried at 60°C until constant weight to determine leaf, stem and leaf:stem ratio.

Hand-plucked samples were used to estimate nutritive value of the grazed portion of the canopy. One sample was taken per pasture every 28d. The samples were plucked to simulate the steers grazing as closely as possible. The grazing behavior of the animals was observed during sampling after prior familiarization. The samples were dried at 60^oC in a forced-air oven to constant weight, ground in a Wiley mill to pass a 1mm stainless steel screen, and taken to the laboratory for analyses.

2.2.2. Animal measurements

Animals were weighted every 28 days. Average daily gain (ADG) was calculated from the initial and final weight, when the weight was reported with curfew of water and feed, whilst the intermediate weights (without curfew) were used to adjust the supplementation levels.

Intake and digestibility estimates were evaluated during ten consecutive days (07/04 – 17/04/2012) in the intermediate phase of the experiment. The technique of three markers (LIPE®, titanium dioxide and indigestible neutral detergent fiber (NDFi), described by Ferreira et al. (2009) was used to estimate forage intake and apparent digestibility. LIPE® (lignin that was isolated, purified, and enriched from *Eucalyptus grandis*, UFMG, Minas Gerais) was used to estimate fecal excretion. During 6 d, 500 mg of the marker was dosed orally to six animals (3 for paddock) from each treatment with one fecal collection per day in alternate hours during the last three days (Santos et al., 2011). Fecal samples were dried (60°C for 72 h), ground (1 mm), and approximately 10g of composite faeces from each animal was sent to the Federal University of Minas Gerais (Universidade Federal de Minas Gerais) for total fecal output estimate, as described by Saliba (2005).

Titanium dioxide was used to estimate the individual intake of concentrate. During 10 d, 10g of the marker was provided per bull, mixed into the concentrate provided to the group. This schedule involved a total of 11 d of dosing, with the last 5 d for the faeces collection, which was performed once a day in alternate hours. Fecal samples were dried (60°C for 72 h), ground (1 mm), and composited in one sample. The samples were analyzed for titanium dioxide, following the technique described by Myers et al., (2004). Concentrate intake was estimated by dividing the fecal excretion of titanium dioxide by its respective concentration in the concentrate.

To estimate the individual forage intake, iNDF was used as an internal marker. For the quantification of iNDF in the samples of faeces, concentrate, and forage, these materials were placed in Ankom filter bags (F57; Ankom Tech. Corp., Fairport, NY) and incubated in the rumen of a fistulated steer for 264 h (Casali et al., 2008). The remaining material from the incubation was washed in water and then subjected to extraction with neutral detergent, with the residue considered to be iNDF. The individual forage intake was estimated by subtracting the excretion of this marker relative to the concentrated from the total excretion of iNDF and dividing this difference by the concentration of iNDF in the forage.

2.3. Laboratory analyses

Samples of concentrated, forage, and faeces were analysed for dry matter (DM), organic matter (OM) and crude protein (CP) (Van Soest & Robertson, 1985). The CP was estimated using the Dumas combustion method, on the Leco®, model FP- 528 (Leco Corporation, Michigan, USA). Ether extract was analyzed by the Soxhlet extraction with petroleum ether. The concentration of NDF corrected for ash and protein was determined by the technique adapted from Mertens (2002) and acid detergent fiber ADF was analyzed using Ankom Tech. Corp., Fairport, NY.

2.4. Statistical analyses

The experimental design for the data analysis was completely randomized in a 3 × 3 factorial design with two replicates. A mixed model was calculated using the MIXED procedure of SAS, version 9.2 (SAS, 2008). First, the best covariance structure was selected using the BIC criterion (Schwarz's Bayesian Criterion). The main effects of forage height, supplementation strategies and periods, and the interactions between these factors, when determined to be significant by analysis of variance, were analyzed using Tukey's post-hoc test at 5% probability; for this analysis, the LSMEANS procedure of SAS was used. For the interactions, the SLICE option of SAS was used, and the experimental periods were considered as a factor of division.

3. RESULTS

The canopy height achieved the target for all treatments; the heights were 15.7±1.1 for the lower height; 25.5±0.9 to the medium and 34.9±0.6 for the tall height. There was difference in the pastures variables only between height without effect on supplement. Herbage mass (HM) leaf mass (LM) stem mass (SM) and dead material (DM) increased with the rise of canopy height (Table 3) without effect on ration L/S.

Stock rate (SR) ranged between canopy heights (Table 3). Pasture maintained on 15 cm showed high SR (5.93 UA/ha) whilst this value decreased when increased the height, 25 cm (4.54 UA/ha) and 35 cm (3.43 UA/ha).

Table 3: Herbage mass (HM) and canopy characteristics of Brachiaria *brizanha* cv. Marandu pasture handled in different heights during wet season

Variables	Heights (cm)			L	Q
	15	25	35		
HM	4364	7588	9614	<0.001	0.387
LM	1394	2160	2845	<0.001	0.952
SM	1267	2324	2977	<0.001	0.251
DM	1712	3108	3779	<0.001	0.013
L/S R	1.07	0.98	0.94	0.639	0.469
SR	5.93	4.54	3.43	<0.001	0.683

LM leaf mass (kg/ha); SM steam mass (kg/ha); DM dead material (kg/ha); R L/S ratio of leaf:steam, SR stock rate (UA/ha) (UA= 450kg BW);
[a,b,c,d]Means within a row with differing superscripts differ (Tukey's test, *P* < 0.05).

Table 4: Dry matter intake (DMI) of young Nellore bulls grazing *Brachiaria* pasture managed in different canopy height and receiving different supplement with soybean differentially available in the rumen during wet season

Variable	Height			SE	Effect		Supplement			SE
	15	25	35		L	Q	C	WSG	PF	
kg/d	6,3	7,2	7,1	0,26	0,061	0,097	5,7^{b}	7,3^{a}	7,6^{a}	0,26
% BW	2,02	2,12	2,06	0,07	0,13	0,446	1,82^{b}	2,17^{a}	2,20^{a}	0,07
CP	0,96	1,05	0,98	0,03	0,603	0,06	0,78^{b}	1,09^{a}	1,12^{a}	0,03
NDF	3,34	3,75	3,69	0,15	0,099	0,199	3,24^{b}	3,6a^{b}	3,95^{a}	0,15

C control (animal received mineral salt); WSG supplement with whole soybean grain; PF supplement with protect fat;
^{a,b,c}Means within a row with differing superscripts differ (Tukey's test, $P < 0.05$).

Table 5: Apparent digestibility by young Nellore bulls grazing Brachiaria brizantha cv. Marandu pasture handled in different canopy height and receiving different supplement with soybean differentially available in the rumen during wet season

Supplement	Canopy heights			Effect	
	15	25	35	L	Q
Dry matter (g/kg)					
C	653.2^{b}	687.8^{b}	660.8	0,426	0,001
WSG	681.7^{a}	735.7^{a}	669.5	0,207	<0,001
PF	697.8^{a}	708.5^{ab}	677.5	0,079	0,041
SE	0,98	0,98	0,98	0,98	0,98
Neutron detergent fiber (g/kg)					
C	620.5^{b}	702.3	670.3	0,060	0,019
WSG	658.3^{ab}	677.0	670.1	0,470	0,371
PF	698.6^{a}	691.1	659.3	0,003	0,240
SE	1,84	1,84	1,84	1,84	1,84

C treatment with mineral salt, WSG supplement with whole soybean grain, PF supplement with protect fat

^{a,b,c}Means within a column with differing superscripts differ (Tukey's test, *P* < 0.05).

The chemical composition did not differ between canopy height and supplement. The averages were 131 g/kg of crude protein; 579 g/kg of neutral detergent fiber; 261 g/kg of acid detergent fiber and 567 g/kg of *in vitro* dry matter digestibility. Increased canopy height reflected in enhance (P=0,061) of dry matter intake (kg/d). The supplemented animals showed greater DMI (kg/d and %BW) whilst difference did not were found in CP and NDF intake for both, height and supplement (Table 4).

Table 6: Performance of young Nellore bulls grazing *Brachiaria brizantha* cv. Marandu pasture handled in different canopy height and receiving different supplement with soybean differentially available in the rumen during wet season

Variable	Height			SE	Effect		Supplement			SE
	15	25	35		L	Q	C	WSG	PF	
IBW	244	243	242	5,08	0,79	0,91	243	244	242	5,08
FBW	312	340	342	6,25	<0,01	0,08	308^{a}	336^{b}	350^{c}	6,25
ADG	0,59	0,86	0,88	0,03	<0,01	0,01	$0,57^{c}$	$0,81^{b}$	$0,95^{a}$	0,03

IBW: initial body weight; FBW: final body weight; ADG: average daily gain.

Apparent digestibility was influenced by height and supplement. The highest and lowest values of dry matter digestibility were recorded in pasture managed in 25 cm with WSG (735.7 g/kg) and 15 cm with mineral salt (653.2 g/kg), respectively. The strategic supplementation increased digestibility in the 15 and 25 cm of canopy height (Table 5). Pasture managed in 25 cm with mineral salt showed the highest NDF digestibility whilst the lower value was achieved by 15 cm of pasture.

Average daily gain varied with canopy height being that swards managed at tall height resulted in greater ADG (0.88 kg/d). The ADG as well was effect by supplements. Animals receiving PF showed the highest value (0.95 kg/d) while animals with mineral salt achieved lower performance (0.57 kg/d).

4. DISCUSSION

The herbage mass decreased linearly from 9.6 to 4.3 Mg ha^{-1} whilst stocking rate increased (42%) from low to tall height (Table 3). The canopy height also affected the morphological components. The effect of stocking rate on HM has been reported in the literature, and a decrease in HM with greater stocking rate is a consequence of increased total forage intake of a greater number of animals. Likely the absence of effect of supplementation on herbage responses were due adjust stocking rate to height target. Da Silva et al., (2013) reported that sward height is the main measurement adopted to manage tropical pastures due to its practicability.

Grazing pressure is one of the most important factors determining the pasture performance. Stocking rate seems the key of management variable influencing productivity of grazing systems (FALES et al., 1995) because it determines herbage mass (BURNS et al., 2002; SOLLENBERGER et al., 2005; SOLLENBERGER and VANZANT, 2011). By this sense, forage mass is a fundamental measure of a beef production system with emphases to increasing leave mass.

The leave and steam mass was 51 and 57 %, respectively, highest in tall height than lower height whilst no difference was showed in L/S ratio. The reason for increasing proportion of plant was likely because of greater herbage accumulation rate. However, dead material also increasing in higher pasture, likely because excess herbage mass may result in self-shading, accumulation of nonphotosynthetic residue, and reduced photosynthesis, especially on the young basal tiller (ADJEI et al., 1980; PARSON et al., 1988; HERNADEZ GARAY et al., 2004).

Differences in chemical composition between canopy heights were expected, however, this lack of difference likely occurred in part due the lower rainfall during the wet season. Forage chemical composition and digestibility are influenced by plant age, and as rest or regrowth period increases, forage matures and the ratio between leaves and stems is reduced (MINSON, 1990).

The ability of a grazing animal to harvest herbage appears the most important factor limiting intake when intake is responding steadily to increases in bite mass (POPPI et al. 1987). The forage quality is usually higher in leaves than in stems and it is generally accepted that animals select leaves and avoid stems (HODGSON et al., 1994). By this sense, tall pastures in this trial allowance high quantity of leaf what likely interfering positively the process of bite formation and affecting bite dimensions and selectivity (BENVENUTTI et al., 2006).

Variation in herbage bulk density may contribute independently to bite mass (HODGSON et al. 1994). Although variation in sward height generates a wider range in intake bite mass, intake tends to increase asymptotically responding to sward height. This is influenced by sward structure and grazing behavior (diet selection and grazing time), characteristics that are strongly influenced by grazing management practices. As shown by Sollenberger and Vanzant (2011) when pastures present a range in forage mass, the variation in ADG can be explained by the quantity.

According Jenking and Palmquist (1984) a negative effect on intake of rich sources of unsaturated fatty acids, such as soybean grain, have effects on the microbial membrane permeability, primarily by inhibiting the gram-positive bacteria activity, where this alter the rumen fermentation (NAGAJARA et al., 1997). Thus, adhesion and multiplication of the cellulolytic bacteria population responsible for fiber fermentation is prevented, thereby reducing feed passage through the digestive system and, consequently, intake. However, greater DMI was observed in the present trial, indicating that supplement treatment did not affect negatively forage intake of young bulls.

Pasture managed in 25 cm showed the highest dry matter apparent digestibility. In generally animals achieve a higher diet quality by selecting more nutritious plant parts. According Benvenutti et al., 2006, there is an increasing body of evidence that shows that the tensile resistance of the stems is a better indicator of plant part selectivity within tropical grasses species that the chemical composition. Likely pasture in 25 cm has stems with low tensile resistance than 35 cm resulting in bigger digestibility.

By other hand, supplemented animals showed high digestibility than treatment with mineral salt. Decrease in digestibility was expected, being which the main problem of using lipids rich in unsaturated fatty acids in diets for ruminants is their effect on intake, digestibility and, consequently, on productivity performance. However, animals receiving 0.5% of BW of supplement in this experiment showed higher apparent digestibility. Furthermore, Allen (2000) suggested that metabolic factors are related to reduction in intake, whereas the ruminal digestibility of the fibrous fraction is acutely affected by the use of unsaturated lipids in diet, yet supplements not affected digestibility on the present experiment.

Average daily gain is associated with intake and digestibility of nutrients (REIS et al., 2013). As a consequence with the rise of canopy height increased animal performance was achieved. The ADG response was attributed to increasing HM with decreasing stocking rate. This conclusion is based on the majority of literature, which shows that across a wide range of stocking rate the ADG response to stocking rate is determined by herbage-quantity-related factors like HM (SOLLENBERGER and

VANZANT, 2011). Bigger difference was showed from 15 cm to 25 and 35 cm, likely because lower stocking rate increased the opportunity for young bulls to select plant part with greater nutritive value and not affected bite mass. However, the lack of effect between 25 and 35 cm on animal performance (20 g) were reported by Poppi et al., (1987) where showed the relationship of pasture intake to level of pasture available to an animal. There are two quite distinct sections of the curve non-nutritional and nutritional factors. In the ascending part of the curve, the ability of the animal to harvest pasture (non-nutritional) appear to be most important in limiting intake. These factors are influenced by pasture structure and the grazing behavior of the animal, and include diet selection, grazing time, bite size and rate of biting. In this part of the curve intake is very sensitive to changes in the amount of pasture allowance, so any errors have a big effect on animals performance what likely has effect performance of animals grazing 15 cm of pasture.

By other hand, at the plateau or asymptotic section of the curve, nutritional factors such as digestibility, the time feed stays in the rumen and concentration of metabolic products appear to be important in controlling intake and consequently average daily, by this regards, likely, no structural difference was found between 25 and 35 cm of height to decrease animal intake, that despite the herbage mass be 21% higher in pasture managed in 35 cm did not reflected in bigger animal performance.

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).

By this sense, animals receiving 5 g/kg of supplement increased performance. The diet containing PF showed greater value (40% bigger than control treatment) which can be explained by the presence of calcium salts in this source, what likely involve of UFA in this supplement which is greater than soybean grain.

The current literature is not conclusive regarding the effects of protected fat on nutrient intake and animal performance. Allen (2000) developed equations involving 24 studies on protected fat reduce the DM intake by 2.5 g/kg. Other studies reported no effect with the supplementation of protected fat on the DM intake of cows and sheep (PALMQUIST, 1991; PEREZ ALBA et al., 1997).

According to McNiven et al., (2004), soybeans are rich in unsaturated fatty acids and are used in diets to increase the amount of lipids, which increases the flow of UFAs into the small intestine. According to Martinez Marin (2007) and Fiorentini et al., (2012), approximately 20% of UFA ingested by ruminants reach the small intestine without undergoing complete biohydrogenation. However, increased or decreased UFA flow into the small intestine depends on the lipid source used and the diet composition (JORDAN et al., 2006).

Moreover, Fiorentini (2013) reported an 80% decrease in ruminal protozoa when animals were fed diets with soybean oil and whole soybean grain compared to diets containing PF. Because they are important producers of H_2 in the rumen, the decreased amount of protozoa reduced the availability of substrate for the metabolism of methanogens, thereby reducing CH_4 (MARTINS et al., 2010) should be expected and consequently lower quantity of energy will be waste reflecting in higher animal performance.

5. CONCLUSION

There is no interaction between canopy height and supplement. Pasture managed in 35 cm showed highest herbage mass and animal performance, however lower stocking rate. Supplementation with protect fat achieved high performance. Beef cattle production systems using tropical forage in the wet season should consider grazing intensity and supplementation in order to provide high productivity.

6. LITERATURE CITED

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