University of São Paulo
"Luiz de Queiroz" College of Agriculture

Rotational stocking management on elephant grass for dairy cows: grazing strategies, animal productivity, enteric methane and nitrous oxide emissions

Guilhermo Francklin de Souza Congio

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

Piracicaba
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To my parents Francklin Roberto Leite Congio (in memorian) and Neuza Aparecida de Souza Congio,
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CONTENTS

RESUMO

Pastejo rotativo em capim-elefante para vacas leiteiras: estratégias de pastejo, produtividade animal, emissões de metano entérico e de óxido nitroso

Sistemas baseados no uso de pastagens são importantes fornecedores de leite para a indústria de latícinios e, dessa forma, terão papel relevante para suportar a crescente demanda por alimentos. No entanto, essa oferta adicional de leite deve ser obtida através de maiores produtividades resultantes da intensificação de sistemas de produção já existentes por meio de estratégias ambientalmente seguras e economicamente rentáveis em direção à intensificação sustentável. A hipótese central deste estudo foi que estratégias simples de manejo do pastejo podem melhorar a eficiência e, ao mesmo tempo, reduzir os principais impactos ambientais dos sistemas de produção animal em pastagens tropicais. Foram realizados dois experimentos em pastagem de capim-elefante (*Pennisetum purpureum* Schum. Cv. Cameroon) não-irrigada em Piracicaba, SP, Brasil. O objetivo do primeiro experimento foi avaliar a influência de duas metas pré-pastejo (95% e máxima interceptação de luz pelo dossel durante a rebrotação; $IL_{95\%}$ e $IL_{Máx}$, respectivamente) sobre a estrutura do pasto e valor nutritivo da forragem, consumo de matéria seca (CMS), produção de leite, taxa de lotação, emissões de metano entérico (CH_4) de vacas HPB × Jersey, e o fluxo de óxido nitroso dos solos. Os resultados indicaram que a altura pré-pastejo foi maior para $IL_{Máx}$ (≈135 cm) do que $IL_{95\%}$ (≈100 cm) e pode ser usada como um guia de campo confiável para monitorar a estrutura do pasto. O manejo do pastejo com base nos critérios de $IL_{95\%}$ melhorou o valor nutritivo da forragem e a eficiência de pastejo, permitindo maior CMS, produção de leite e taxa de lotação. A emissão diária de CH_4 entérico não foi afetada; no entanto, as vacas que pastejaram o capim-elefante manejado por $IL_{95\%}$ foram mais eficientes e emitiram 21% menos CH_4/kg de leite e 18% menos CH_4/kg de MS consumida. O aumento de 51% na produção de leite por hectare superou o aumento de 29% nas emissões de CH_4 entérico por hectare para a meta $IL_{95\%}$. Os fluxos de óxido nitroso não foram afetados pelas metas pré-pastejo. De maneira geral, o manejo do pastejo com base na meta $IL_{95\%}$ é uma prática ambientalmente segura que melhora a eficiência de uso dos recursos alocados por meio da otimização de processos envolvendo plantas, ruminantes e sua interface, e aumenta a eficiência da produção de leite em sistemas baseados em pastagens tropicais. Uma vez que a meta pré-pastejo ideal foi estabelecida durante o primeiro experimento ($IL_{95\%}$), a segunda etapa consistiu-se em um refinamento da primeira. O segundo objetivo foi descrever e medir a influência de dois horários de alocação de novos piquetes aos animais (AM e PM) sobre a composição química da forragem, CMS, produção e composição do leite, e emissões de CH_4 entérico de vacas HPB × Jersey. Os resultados confirmaram a compreensão geral da variação diurna na composição química da forragem em direção a maiores concentrações de matéria seca e de carboidratos não-fibrosos, e menor concentração de componentes da fibra na forragem amostrada pela à tarde. No entanto, o maior valor nutritivo da forragem da tarde não aumentou o CMS e a produção de leite, nem diminuiu a intensidade de emissão de CH_4 das vacas leiteiras. Os resultados também indicaram que a alocação à tarde pode ser uma estratégia de manejo simples e útil que resulta em maior partição de N para produção de proteína, e menor excreção de N ureico no leite. A associação da meta pré-pastejo $IL_{95\%}$ e a alocação do rebanho para um novo piquete à tarde poderia trazer benefícios econômicos, produtivos e ambientais para a intensificação sustentável de sistemas baseados em pastagens tropicais.

Palavras-chave: Gases de efeito estufa; Gramínea tropical; Interceptação luminosa do dossel; Manejo do pastejo; Qualidade da forragem

ABSTRACT

Rotational stocking management on elephant grass for dairy cows: grazing strategies, animal productivity, enteric methane and nitrous oxide emissions

Pasture-based systems are important milk suppliers to dairy industry and thereby will play relevant role to support the growing demand for food. However, this additional milk supply must be obtained through higher yields resulting from intensification of existing farming systems through strategies environmentally friendly and economically profitable towards sustainable intensification. The central hypothesis of this study was that simple grazing management strategies can improve the efficiency while reduce the key environmental issues of tropical pasture-based dairy systems. Two experiments were carried out on a rainfed and non-irrigated elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon) pasture in Piracicaba, SP, Brazil. The objective of the first experiment was to investigate the influence of two pre-grazing targets (95% and maximum canopy light interception during pasture regrowth; $LI_{95\%}$ and LI_{Max}, respectively) on sward structure and herbage nutritive value, dry matter intake (DMI), milk yield, stocking rate, enteric methane (CH_4) emissions by Holstein × Jersey dairy cows, and nitrous oxide fluxes from the soil. Results indicated that pre-grazing canopy height was greater for LI_{Max} (≈135 cm) than $LI_{95\%}$ (≈100 cm) and can be used as a reliable field guide for monitoring sward structure. Grazing management based on the $LI_{95\%}$ target improved herbage nutritive value and grazing efficiency, allowing greater DMI, milk yield and stocking rate by dairy cows. Daily enteric CH_4 emission was not affected; however, cows grazing elephant grass at $LI_{95\%}$ were more efficient and emitted 21% less CH_4/kg of milk yield and 18% less CH_4/kg of DMI. The 51% increase in milk yield per hectare overcame the 29% increase in enteric CH_4 emissions per hectare for the $LI_{95\%}$ target. Nitrous oxide fluxes were not affected by pre-grazing targets. Overall, strategic grazing management is an environmentally friendly practice that improves the use efficiency of allocated resources through optimization of processes involving plant, ruminant and their interface, and enhances milk production efficiency of tropical pasture-based systems. Once the ideal pre-grazing target was established during he first experiment ($LI_{95\%}$), the second step consisted of a refinement of the first phase. The second objective was to describe and measure the influence of two timings of new paddock allocation to cows (AM and PM) on herbage chemical composition and DMI, milk yield, milk compostion, and enteric CH_4 emissions of Holstein × Jersey dairy cows. Results supported the general understanding of diurnal variation in herbage chemical composition towards greater concentrations of dry matter and non-fibrous carbohydrates, and lower concentration of fiber components in the afternoon herbage. However, the higher nutritive value of the afternoon herbage did not result in increasead DMI and milk yield, or decreased intensity of CH_4 emission by dairy cows. Our findings also indicate that new paddock allocation in the afternoon can be a simple and useful grazing strategy that results in greater N partitioning to protein yield, and lower excretion of urea N in milk. The association of $LI_{95\%}$ pre-grazing target and PM allocation could bring economic, productive and environmental benefits towards sustainable intensification of tropical pasture-based systems.

Keywords: Greenhouse gases; Tropical grass; Canopy light interception; Grazing management; Herbage quality

1. INTRODUCTION

To meet the world's future food demand, agricultural outputs must grow from 60 to 120% by 2050 (Godfray et al., 2010; Conforti, 2011; Alexandratos and Bruinsma, 2012) while agriculture environmental footprint must decrease dramatically (Foley et al., 2011). Therefore, food producers are faced with the challenge of supplying food demand through environmentally friendly (Tilman et al., 2002) and economic favorable practices (Foote et al., 2015; Gregorini et al., 2017). In developing countries, agriculture production must increase 80% through higher yields resulting from intensification of existing agricultural systems (Conforti, 2011). Sustainable intensification was defined as a form of production wherein yields are increased without adverse environmental impact and without the cultivation of more land (Royal Society, 2009). Despite contested (Struik and Kuyper, 2017), this term was deeply discussed (Pretty and Bharucha, 2014) and highlights the needs to increase the productivity (i.e. agricultural product outputs per hectare) of current agricultural systems through practices that minimize key environmental issues (Garnett and Godfray, 2012).

Global warming observed since the mid-20th century is mostly attributed to anthropic activities that emit greenhouse gases (GHG; IPCC, 2014). Agricultural systems contribute with 10-12% of global estimated GHG emissions, 50% of methane (CH_4) and 60% of nitrous oxide (N_2O) from anthropogenic sources (Smith et al., 2007). Dairy farming systems provide essential high-quality protein that is a major component of human diet (O'Brien et al., 2015; Aguirre-Villegas et al., 2017). However, considering livestock production, they are the second largest contributor accounting for 20% of total GHG emissions (Gerber et al., 2013). Life cycle assessment approaches reported enteric CH_4 and N_2O from soils as predominant sources of GHG in dairy farming systems, representing approximately 90% of total GHG emissions (Aguirre-Villegas et al., 2017). In tropical dairy farming systems, Cunha et al. (2016) reported 53% for enteric CH_4 and 18% for N_2O of total GHG emissions for typical Brazilian dairy farms.

Pasture-based systems are important milk suppliers to dairy industry in temperate (Chapman, 2016; Macdonald et al., 2017) and tropical climate (Santos et al., 2014) and thereby will play relevant role to support growing demand (Godfray et al., 2010; Conforti, 2011; Alexandratos and Bruinsma, 2012). The intensification of temperate pasture-based dairy systems has been associated with increasing inputs such as nitrogen fertilizer or imported supplements (Beukes et al., 2012; Foote et al., 2015; Macdonald et al., 2017). However, such intensification practices are associated with issues of environmental concern, namely increased GHG emissions and water degradation (Foley et al., 2011; Vogeler et al., 2013; Foote et al., 2015). Alternatively, grazing management strategies that optimize herbage utilization and digestible dry matter intake by grazing cows could improve land-use and decrease GHG emissions of pasture-based dairy systems (Muñoz et al., 2016; Gregorini et al., 2017).

The key to understanding the principles of grazing management strategies is to comprehend that the harvestable components are photosynthetic organs – predominantly leaves (Parsons et al., 2011). Studies have reported that grazing management strategies that prioritize leaf accumulation rather than other plant-part components may be useful tools towards efficient pasture-based systems in the tropics (Silveira et al., 2013; Pereira et al., 2014; Da Silva et al., 2015; Da Silva et al., 2017; Sbrissia et al., 2018). Leafy swards mean high herbage quality, since it provides high short-term intake rate by grazing animals, as leaves require less strength to be harvested, and also because they have greater nutritive value than stems and dead material (Trindade et al., 2007; Silva, 2017). In this sense, the development of efficient pasture-based systems with perennial tropical grasses usually focuses on the control of stem elongation and excessive senescence and dead material accumulation by grazing management strategies (Da Silva and Carvalho, 2005; Da Silva et al., 2015).

Although the studies aforementioned have demonstrated the benefits of grazing management strategies, most focused solely on plant responses. There is a knowledge gap relating plant and animal responses and environmental benefits in tropical pasture-based dairy systems. Therefore, the central objective of this study was to investigate the influence of simple grazing management strategies and their effects on the relationships among plant, animal and soil components. The central hypothesis was that simple grazing management strategies optimize processes inherent to plant growth, plant-animal interface, and animal, and provide environmental services, improving efficiency of tropical pasture-based system.

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2. LITERATURE REVIEW

2.1. Grazing management and herbage characteristics

The pasture management concept involves a wide range of aspects such as the choice of the ideal forage species or mix, liming, nutrient balance and fertilization rate, weed and pest management, soil conservation practices, paddock subdivision, watering system, type and level of supplementation, among others. On the other hand, grazing management is a specific term that refers to monitoring the sward state and controlling the grazing process by grazers through targets that optimize herbage regrowth and animal responses (Da Silva and Corsi, 2003). In continuous stocking grazing management strategies, the question would be at which sward surface height (SSH) the grazer should keep the herbage in order to balance sub-optimal plant and animal responses? In intermittent grazing management strategies (i.e. rotational grazing) the question would be which are the most adequate pre- and post-grazing heights to achieve the same goals?

Rotational stocking management is widely used in temperate grazing systems and is also being adopted in tropical conditions mainly in dairy farming systems (Santos et al., 2014; Chapman et al., 2016). A large number of studies have been developed to try and understand the most adequate combination between frequency and severity of defoliation for several tropical forage species, or the ideal pre- and post-grazing heights (i.e. frequency and severity, respectively) (Carnevalli et al., 2006; Barbosa et al., 2007; Trindade et al., 2007; Da Silva et al., 2009; Difante et al., 2009a; Difante et al., 2009b; Giacomoni et al., 2009; Difante et al., 2010; Barbosa et al., 2011; Gimenes et al., 2011; Zanini et al., 2012; Silveira et al., 2013; Geremia et al., 2014; Pereira et al., 2014; Pereira et al., 2015a; Pereira et al., 2015b; Silveira et al., 2016; Da Silva et al., 2017; Pereira et al., 2018). The majority of these studies evaluated frequencies based on canopy light interception (LI) combined with severities based in fixed post-grazing heights, and focused on plant responses such as tillering dynamics, morphogenesis, organic reserves, herbage nutritive value, sward structure, and herbage accumulation. These studies observed that tropical grasses regrowth is a function of canopy LI and leaf area index (LAI) with accumulation of herbage fitted to a sigmoid curve with three distinct phases as proposed for temperate swards by Brougham (1955). During the early stages of regrowth, leaves are the main morphological component accumulated. As LAI increases, canopy light intra-competition increases and plants change their growth pattern as a means of optimizing light capture through stem elongation. The shift in growth pattern occurs when canopy LI reaches and exceeds 95% ($LI_{95\%}$; Da Silva et al., 2015). These studies have shown systematic relationship between SSH and LI, establishing SSH as a reliable field index for monitoring and controlling herbage regrowth (Da Silva et al., 2015).

Grazing management affects the distribution and arrangement of above-ground plant-part components (i.e. sward structure, Laca and Lemaire, 2000). The frequency of defoliation based on $LI_{95\%}$ often minimizes stem elongation of tropical forage, maximizing leaf blade proportion over others sward plant-part components (Carnevalli et al., 2006; Barbosa et al., 2007; Trindade et al., 2007; Da Silva et al., 2009). Studies showed that swards managed with the $LI_{95\%}$ target have greater leaf appearance rate, leaf elongation rate, and leaf accumulation in successive grazing cycles than swards managed with the LI_{Max} target, which have greater stem elongation and senescence (Barbosa et al., 2011; Pereira et al., 2014; Pereira et al., 2015b; Silveira et al., 2016; Pereira et al., 2018). As a result between leaf growth and senescence rates, $LI_{95\%}$ provides greater average net growth rate and is considered the critical LAI to interrupt regrowth under rotational grazing management (Da Silva et al., 2015). Pereira et al. (2015a) also reported changes in horizontal sward structure as a function of grazing management. The $LI_{95\%}$ target provided greater soil cover by elephant grass tussocks (*Pennisetum purpureum* Schum. cv. Napier). Furthermore, the exacerbated competition for light for the LI_{Max} target resulted in tiller death, reduced tillering, and less stability of plant population impairing pasture persistence (Pereira et al., 2015a).

Herbage chemical composition is a function of the proportion of plant-part components in the herbage mass and their tissue anatomy (Moore, 1994). Stems contain higher proportion of cell wall tissues and less photosynthetic tissues than leaves (Wilson and Kennedy, 1996). On the other hand, most protein compounds are present in leaves, with the majority associated with photosynthetic enzymes (Gastal and Durand, 2000). As a consequence of changes in plant-part components in the grazing strata, the frequency of defoliation associated with the $LI_{95\%}$ target is an efficient tool to improve herbage nutritive value in tropical grasses (Trindade et al., 2007). Studies reported lower acid-detergent fiber and greater crude protein concentrations for elephant grass (*Pennisetum purpureum* Schum) (Voltolini et al., 2010a; Geremia et al., 2014), and greater *in vitro* digestible organic matter for signal grass (*Brachiaria decumbens* cv. Basilisk, syn. *Urochloa decumbens* Stapf R. D. Webster) (Pedreira et al., 2017) managed with the $LI_{95\%}$ rather than the LI_{Max} target.

Efficient pasture-based systems should maximize the proportion of consumed relatively to produced herbage (Chapman et al., 2016). In order to do that, they have to prioritize leaf accumulation and increase grazing efficiency or herbage utilization through reduced losses by cattle trampling and plant senescence (Da Silva et al., 2015; Chapman et al., 2016). Several studies have shown greater senescence for tropical grasses managed with the LI_{Max} compared to the $LI_{95\%}$ target because of the longer regrowth intervals (Barbosa et al., 2011; Pereira et al., 2014; Pereira et al., 2015b; Silveira et al., 2016; Pereira et al., 2018). Longer regrowth intervals usually result in taller swards with high pre-grazing herbage mass (Da Silva et al., 2009; Pereira et al., 2015b) which are more susceptible to losses by cattle trampling (Carnevalli et al., 2006; Silveira et al., 2013). Both greater senescence and grazing losses by cattle trampling contribute to dereased grazing efficiency of taller swards managed with the LI_{Max} compared to the $LI_{95\%}$ target.

Studies showing the numerous benefits on plant growth managed with the $LI_{95\%}$ target were mostly compared with management using the LI_{Max} target (early known as $LI_{100\%}$). Recently, Sbrissia et al. (2018) assessing a range of LI targets lower than 95%, highlighted a new opportunity for tropical forage grasses under rotational stocking management. They suggested that there is a range of pre-grazing heights with no impact on net herbage accumulation rate, as long as the defoliation used is moderate (removal of no more than 50% of the initial pre-grazing height). The authors explained that the same homeostatic mechanisms that buffer herbage accumulation across a range of targets in continuously stocked swards can be applied to rotationally stocked swards. If more studies corroborate these responses for different tropical grasses, farmers would have a flexible optimal range to manage their pastures where $LI_{95\%}$ would be the upper threshold to interrupt sward regrowth.

Regarding severity of defoliation, studies that assessed mainly plant responses based on fixed residual post-grazing heights usually observed that greater severities (i.e. lower post-grazing heights) were positively related to herbage accumulation and grazing efficiency, and negatively related to nutritive value of the consumed herbage (Carnevalli et al., 2006; Barbosa et al., 2007; Difante et al., 2009b). However, using the concept of severity of defoliation as a percentage of initial pre-grazing height and having the grazing animal under perspective, studies have shown that levels of defoliation until 40-50% of the pre-grazing height result in relatively stable and high rate of short-term herbage intake (Fonseca et al., 2012; Fonseca et al., 2013; Carvalho, 2013; Mezzalira et al., 2014). They reported that beyond this herbage depletion level preferred leaves become scarce and stem and dead material become predominant in succeeding lower pasture layers impairing the efficiency of nutrient harvesting per unit of bite (Carvalho, 2013). According to Zanini et al. (2012), regardless of forage species and pre-grazing height, 90% of stem is present in the lower half of the canopy.

2.2. Grazing management and animal responses

Defoliation strategies change tissue turnover, photosynthates allocation pattern, and finally the rate of processes related to morphogenetic characteristics that, in turn, determine sward structural characteristics (Chapman and Lemaire, 1993). As detailed in the previous session, the pre-grazing target of $LI_{95\%}$ under rotational grazing management optimizes harvestable plant-part components (i.e. leaves) rather than support morphological components (i.e. stems) and dead material, which are plant-part components avoided by grazers (Trindade et al., 2007). Furthermore, grazing losses by cattle trampling are reduced with grazing at $LI_{95\%}$ compared to LI_{Max}. As a result of greater leaf accumulation and lower losses by trampling and senescence, the $LI_{95\%}$ target provides more feed per hectare supporting higher stocking rates. Voltolini et al. (2010b) and Gimenes et al. (2011) found stocking rate increases ranging from 10% to 42% in elephant and palisade grass pastures managed with the $LI_{95\%}$ relative to the LI_{Max} target.

Daily herbage intake is determined by interactions between sward structure and grazing animals (Wade and Carvalho, 2000). Poppi et al. (1987) suggested that herbage intake follows an asymptotic distribution represented by two distinct phases. In the first ascending phase, herbage intake is related to sward structure (i.e. herbage or leaf mass, pre-SSH, leaf-to-stem ratio) and grazing behavior (i.e. grazing time, diet selection, bite mass and bite rate), which are characteristics strongly affected by grazing management strategies (Da Silva and Carvalho, 2005). In the second asymptotic phase, nutritional factors such as herbage chemical composition, digesta retention time in the rumen and concentration of metabolic compounds are more relevant in controlling intake (Poppi et al., 1987). Swards constantly kept at taller heights (such as those managed with the LI_{Max} target) result in lower short-term intake rate owing to the excessive length of leaf blade and lower bulk density of herbage in the upper strata (Palhano et al., 2007; Fonseca et al., 2013; Carvalho, 2013). At the rumen level, more fibrous herbage (i.e. higher NDF, ADF and lignin) is associated with greater ruminal retention time, lower fermentation and passage rate, and lower herbage intake (Mertens, 1994; Allen, 1996; Allen, 2000; Forbes, 2007). On the other hand, leafy swards with high herbage nutritive value as those resulting from management with the $LI_{95\%}$ target would optimize animal grazing behavior and rumen fill in order to achieve high daily herbage intake. It is worth mentioning that frequencies of defoliation based on fixed-length rest periods in an attempt to easy operationalize the herd management into set-paddock area are unable to adequately control sward structure and usually result in decreased animal performance and animal productivity (Pedreira et al., 2009; Voltolini et al., 2010b; Euclides et al., 2014).

The severity of defoliation can also affect grazing behavior and nutritive value of the consumed herbage (Difante et al., 2009b; Fonseca et al., 2012). Fonseca et al. (2012) reported that severities of defoliation greater than 40-50% removal of pre-grazing height resulted in linear decrease of the short-term rate of herbage intake jeopardizing daily herbage intake and animal performance. At the same time, severities of defoliation greater than the ones proposed by Fonseca et al. (2012) would optimize grazing efficiency and stocking rate (Difante et al., 2009a). Thus, there is a clear trade-off between animal performance and stocking rate as proposed early by Mott (1960), and the most productive grazing strategy should be one able to conciliate significant levels of animal performance with the highest possible stocking rate. Recent approaches have shown that defoliation levels around 45% of pre-grazing height can increase in 68% animal performance coupled with stocking rate reductions of around 30% (Euclides et al., 2015; Euclides et al., 2018). Therefore, grazing management strategies that associate the $LI_{95\%}$ pre-grazing target with moderate levels of defoliation (not exceeding the removal of 50% of the pre-grazing height) seem to be more appropriate to achieve higher levels of animal productivity.

However, at the present time, environmental concern is undividable from successful and productive animal production systems (Chiavegato et al., 2018). Greenhouse gases (GHG) emissions are estimated to be the most significant among all categories of environmental impacts in livestock farming systems (O’Brien et al., 2012; Guerci et al., 2013; Gregorini et al, 2016), and enteric methane (CH_4) represents more than 80% of total GHG emissions in pasture-based dairy farming systems (Aguirre-Villegas et al., 2017). Enteric CH_4 production from animal digestion is affected by the amount and nature of feed, and the extent of its degradation, which in turn determines the amount of hydrogen formed in the rumen (Janssen, 2010). The model proposed by Janssen (2010) suggests that greater digesta passage rates increase hydrogen concentration in the rumen. Consequently, microorganisms would select pathways thermodynamically more favorable to this condition, which produce less hydrogen resulting in less CH_4 formed per unit of feed ingested (i.e. CH_4 yield). Studies carried out in temperate grazing systems have shown that pre-grazing height of typical ryegrass × white clover mixed pastures can be an important tool to mitigate enteric CH_4 emissions from pasture-based farming systems. These studies reported no differences in daily enteric CH_4 emissions from beef heifers (Boland et al., 2013) and dairy cows (Wims et al., 2010; Muñoz et al., 2016) grazing low versus high herbage mass swards, even with significant differences reported in daily herbage intake and herbage nutritive value. However, they observed reductions on CH_4 yield and CH_4 emission intensity (i.e CH_4 per unit of final product) from cows grazing low versus high herbage mass swards (Wims et al., 2010; Boland et al., 2013; Muñoz et al., 2016).

2.3. Grazing management and soil properties

Grazing management strategies can strongly affect processes related to plant growth (Da Silva et al., 2015), animal ingestive behavior (Da Silva and Carvalho, 2005), and soil characteristics (de Klein et al., 2008; Luo et al., 2017). Frequency of defoliation based on the $LI_{95\%}$ target increases leaf accumulation and reduces litter deposition owing to decreased senescence and grazing losses by cattle trampling, compared with the LI_{Max} target. As a consequence, studies have shown that the $LI_{95\%}$ target provides more feed per hectare supporting up to 42% increase in stocking rate (Voltolini et al., 2010b). Higher stocking rates modify soil properties (i.e. bulk density, moisture, temperature, pH, aeration) (Warren et al., 1986; Silva et al., 2003; Schmalz et al., 2013) and increase nitrogen (N) discharge to soil through more frequent deposition of urine and feces patches on paddocks (de Klein et al., 2008). These factors, in turn, change microbial community growth and activity (Bardgett et al., 1996; Bardgett et al., 2001; Bardgett and Wardle, 2003) and determine the intensity of processes associated to nitrous oxide (N_2O) flux derived from soils (de Klein et al., 2008; Levine et al., 2011; Luo et al., 2017).

Nitrous oxide is the main GHG from soil and the second most representative between all GHG, ranging from 15% (housed) to 25% (pasture-based) of total GHG emissions in dairy farming systems (Aguirre-Villegas et al., 2017). Nitrous oxide is formed through microbial transformation of N compounds in the soil, typically by incomplete denitrification or by nitrification (Wrage et al., 2001; Saggar et al., 2013). Nitrification is an aerobic process where soil microbials oxidise NH_4^+ to NO_3^- and N_2O is formed through chemical decomposition of intermediates, while denitrification is an anaerobic process where NO_3^- is reduced into N_2, with N_2O an obligatory intermediate (Wrage et al., 2001; de Klein and Eckard, 2008). Nitrous oxide fluxes are affected by a wide range of proximal and distal regulators, making its regulation a very complex process (de Klein et al., 2008; Luo et al., 2017). Proximal soil factors include mineral nitrogen (NH_4^+ and NO_3^-) and organic carbon availabilities, moisture, pH, temperature, and texture that are, in turn, affected by distal regulators such as rainfall or irrigation, soil compaction, organic matter and N inputs (de Klein et al., 2008; Luo et al., 2017). In grazed pastoral soils, the key drivers related to N_2O fluxes are N inputs (i.e. excreta and fertilizer) and soil aeration (i.e. water-filled pore space, WFPS) (de Klein et al., 2008; Luo et al., 2017). Periods when soil characteristics favorable to N_2O production coincide are called “hot moments” (Luo et al., 2017). In tropical conditions, these “hot moments” usually occur during late spring and summer when pastures are intensively growing owing to the abundance of solar radiation, rainfall, and N inputs.

The majority of studies involving N_2O flux from pasture soils have assessed the effects of proximal factors on processes and emission factors in temperate conditions (Saggar et al., 2013; de Klein et al., 2014; Barneze et al., 2015; Venterea et al., 2015; Gardiner et al., 2016; Samad et al., 2016; Clough et al., 2017; Gardiner et al., 2017; van der Weerden et al. 2017; Luo et al., 2018; Rex et al., 2018). The little information available for tropical pastures has also focused on nitrous oxide fluxes related to proximal factors within urine patches (Barneze et al., 2014; Lessa et al., 2014; Mazzetto et al., 2014; Mazzetto et al., 2015). There is no information available regarding N_2O fluxes from soils of tropical pasture-based dairy farming systems, as influenced by grazing management strategies. In fact, farming scale studies are scarce even in temperate conditions. Previous results have shown that intensively managed grasslands are stronger sources of N_2O than extensively managed grasslands owing to greater inputs of N fertilizer and excreta (Smith et al., 2001; Flechard et al., 2007; Rafique et al., 2011). However, they have not accounted for animal outputs that are usually greater in intensively managed systems and could compensate the higher N_2O fluxes.

2.4. Diurnal variation in herbage chemical composition and its implications to pasture-based animal production systems

Several studies have reported diurnal variations in herbage chemical composition (Lechtenberg et al., 1971; Orr et al., 1997; Ciavarella et al., 2000; Griggs et al., 2005; Gregorini et al., 2006; Shewmaker et al., 2006; Gregorini et al., 2008; Morin et al., 2011; De Oliveira et al., 2018). Such variation is mainly related to the balance between the photosynthesis and respiration processes coupled with water loss through plant transpiration (Gregorini, 2012). Photosynthetic activity occurs in chloroplasts mainly in the leaves, and when synthesis of carbohydrates exceeds their use the surplus may be temporarily stored in organs present in leaves and stems (Perry and Moser, 1974; Parsons et al., 1983). Sucrose and fructans are the predominant carbohydrate constituents of temperate grasses (i.e. C_3 metabolism), while sucrose and starch are typical in tropical grasses (i.e. C_4 metabolism;

White, 1973; Chatterton et al., 1989; Pollock and Cairns, 1991). The surplus carbohydrate stored inside the chloroplasts during the day is known as transitory and is used as a source of carbon to plant respiration at night (Lu et al., 2005; Zeeman et al., 2007; Weise et al., 2011).

The balance between these processes leads to non-fibrous carbohydrate (NFC) and dry matter (DM) concentration increases from dawn to dusk, reaching greatest concentrations between 12-13h after sunrise (Lechtenberg et al., 1971; Morin et al., 2011; Morin et al., 2012; De Oliveira et al., 2018). The increase of NFC occurs mainly in the upper layers of sward owing to greater proportion of leaves rather than other plant-part components (Delagarde et al., 2000; De Oliveira et al., 2018). For temperate swards, including grass and legumes, Pelletier et al. (2010) reported increases of soluble carbohydrates (SC) from 6 to 105% for PM herbage compared to AM herbage; however, most results reported mean increases around 50% (Ciavarella et al., 2000; Mayland et al., 2000; Pelletier et al., 2010; Vasta et al., 2012; Pulido et al., 2015; Vibart et al., 2017). Increases in starch have been reported around 100% for PM temperate legumes (Orr et al., 1997; Brito et al., 2008; Pelletier et al., 2010; Andueza et al., 2012) and 30% for PM temperate grass swards (Orr et al., 1997; Bertrand et al., 2008; Pelletier et al., 2010; Brito et al., 2016). Regarding DM concentration, most literature reported increases from 14 up to 27% (Ciavarella et al., 2000; Delagarde et al., 2000; Trevaskis et al., 2001; Gregorini et al., 2008; Abrahamse et al., 2009; De Oliveira et al., 2014; Pulido et al., 2015; Vibart et al., 2017). Gregorini et al. (2009) explained that the diurnal changes in temperature, solar radiation, and relative humidity, coupled with accumulation of photosynthates may explain the increase in DM concentration from AM to PM.

The increase in NFC and DM concentrations during the day often dilutes other nutritional entities (Gregorini et al., 2009; Gregorini, 2012; Vibart et al., 2017). Studies have reported decreases in fiber concentration (Orr et al., 2001; Burns et al., 2007; Abrahamse et al., 2009) associated to greater digestibility (Burns et al., 2007; Pelletier et al., 2010) for PM temperate swards. Considering protein fractions, studies have reported decrease for PM compared to AM herbage (De Oliveira et al., 2014; Pulido et al., 2015; Vibart et al., 2017) while other showed no effect (Gregorini et al., 2008; Delagarde et al., 2000; Fisher et al., 2002). In fact, greater concentrations of SC and starch in the afternoon improve the NFC/protein ratio which optimize the supply of energy and protein to rumen microorganisms (Bryant et al., 2012; Bryant et al., 2014) reducing urinary-N discharges onto pastures (Gregorini et al., 2010; Gregorini, 2012; Vibart et al., 2017). Moreover, Gregorini et al. (2009) observed that diurnal increases of herbage DM and NFC concentrations, associated with dilution of fiber concentration diminished leaf toughness and reduced particle size from AM to PM. The fluctuations in chemical composition, toughness and particle size mean that herbage feeding value (i.e. herbage quality) is highest during the afternoon and early evening (Gregorini, 2012).

Daily herbage intake of grazing ruminants is described by the cumulative outcome of all meals (i.e. grazing events) during the day (Gibb, 2007). Studies have reported three to five grazing events during cooler parts of the day (Gregorini, 2012). However, regardless of the frequency, the major grazing events occur early in the morning and late in the afternoon/early evening for sheep (Orr et al., 1997), beef cattle (Gregorini et al. 2007), and dairy cows (Gibb et al. 1998). The temporal pattern of herbage intake, ingestive and digestive behavior of grazing ruminants can be altered by timing of animal allocation to new strips or paddocks when subjected to intermittent stocking management (Gibb et al., 1998; Orr et al., 2001; Gregorini et al., 2006; Gregorini et al., 2008; Abrahamse et al., 2009; Gregorini, 2012; Pulido et al., 2015; Vibart et al., 2017). When a new paddock or strip is allocated during the afternoon, ruminants display fewer, longer and more intensive grazing bouts in late afternoon and early evening compared with daily morning allocation (Orr et al., 2001; Gregorini et al., 2006; Gregorini et al., 2008; Abrahamse et al., 2009). Although these studies have not reported that the observed shifts can increase daily herbage intake, Gregorini (2012) suggested that ruminants moved to new fresh paddocks or strips in the afternoon may have increased nutrient intake owing to longer grazing periods when herbage quality is at its peak, resulting in average increases of 5% in daily milk yield (Orr et al., 2001; Abrahamse et al., 2009; Mattiauda et al., 2013; Pulido et al., 2015; Vibart et al., 2017).

According to Janssen (2010), the nature and amount of feed are key determinants of enteric CH_4 emissions from ruminants. Therefore, diurnal variations in herbage chemical composition associated with afternoon paddock allocation could be a strategy to mitigate enteric CH_4 emissions of livestock pasture-based systems. Modeling results have shown reductions in enteric CH_4 emissions intensity (g/kg of milk) by dairy cows when herbage NFC increases at the expense of fiber content (Ellis et al., 2012), and Gregorini (2012) suggested the need of field research to assess this hypothesis.

2.5. Conceptual model, objectives and hypotheses

Based on literature review, a conceptual model was created aiming at integrating the relationships among plant, animal and soil components as a function of grazing strategies in tropical pasture-based dairy systems (Figure 1). The objective was to assess and understand the effect of two strategies of rotational grazing management (light blue boxes; Figure 1) on plant and animal responses, and soil parameters in an intensive tropical pasture-based dairy system.

For the first phase of the study, the central hypothesis was that changing sward structure through strategies of rotational grazing management would optimize processes related to plant growth (green boxes), plant-animal interface and animal responses (yellow boxes) which would in turn, affect soil parameters that determine processes associated to N_2O flux from soils (orange boxes). The objective was to describe and measure the influence of two pre-grazing targets ($LI_{95\%}$ and LI_{Max}) on herbage accumulation of elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon), milk outputs of Holstein × Jersey cows, and soil parameters (N_2O emission and WFPS, soil NH_4^+ and soil NO_3^-).

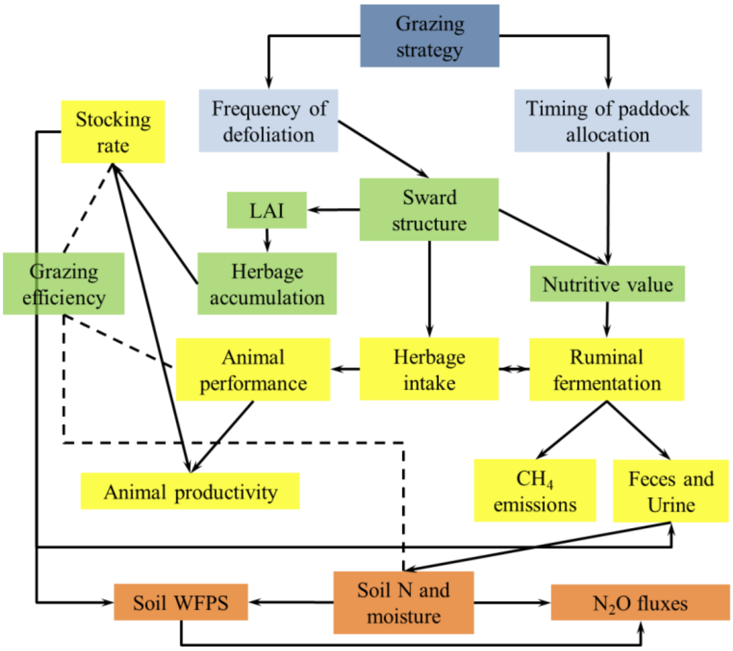

Figure 1. Conceptual model - *Blue boxes: controlled factors (treatments); green boxes: plant responses; yellow boxes: animal responses; orange boxes: soil parameters*

Once the ideal pre-grazing target ($LI_{95\%}$ or LI_{Max}) was established during the first phase, the second step consisted of a refinement of the first phase. The hypothesis was that the diurnal variation in herbage chemical composition of elephant grass coupled with afternoon allocation of the herd to a new fresh paddock would increase nutrient intake and milk outputs, and decrease the intensity of enteric CH_4 emission of Holstein × Jersey cows. The objective was to describe and measure the influence of two timings of paddock allocation (AM and PM) on elephant grass herbage chemical composition and milk outputs of Holstein × Jersey cows.

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3. STRATEGIC GRAZING MANAGEMENT TOWARDS SUSTAINABLE INTENSIFICATION AT TROPICAL PASTURE-BASED DAIRY SYSTEMS[1]

Abstract

Agricultural systems are responsible for environmental impacts that can be mitigated through the adoption of more sustainable practices. The objective of this study was to investigate the influence of two pre-grazing targets (95% and maximum canopy light interception during pasture regrowth; $LI_{95\%}$ and LI_{Max}, respectively) on sward structure and herbage nutritive value of rotationally grazed elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon), and dry matter intake (DMI), milk yield, stocking rate, enteric methane (CH_4) emissions by Holstein × Jersey dairy cows. It was hypothesized that grazing strategies can modify sward structure and improve the nutritive value of the consumed herbage, increasing DMI and reducing the intensity of enteric CH_4 emissions, providing environmental and productivity benefits to tropical pasture-based dairy systems. Results indicated that pre-grazing sward surface height was greater for LI_{Max} (≈135 cm) than $LI_{95\%}$ (≈100 cm) and can be used as a reliable field guide for monitoring sward structure. Grazing management based on $LI_{95\%}$ criterion improved herbage nutritive value and grazing efficiency, allowing greater DMI, milk yield and stocking rate by dairy cows. Daily enteric CH_4 emission was not affected; however, cows grazing elephant grass at $LI_{95\%}$ were more efficient and emitted 21% less CH_4/kg of milk yield and 18% less CH_4/kg of DMI. The 51% increase in milk yield per hectare overcame the 29% increase in enteric CH_4 emissions per hectare in $LI_{95\%}$ grazing management. Thereby the same resource allocation resulted in a 16% mitigation of the main greenhouse gas from pasture-based dairy systems. Overall, strategic grazing management is an environmentally friendly practice that improves the use efficiency of allocated resources through optimization of processes involving plant, ruminant and their interface, and enhances milk production efficiency of tropical pasture-based systems.

Keywords: Canopy light interception; Enteric methane emissions; Herbage quality; Land-use improvement; Milk production efficiency; Elephant grass

3.1. Introduction

To meet the world's future food demand and environmental needs, agricultural outputs must grow from 60 to 120% (Godfray et al., 2010; Conforti, 2011; Alexandratos and Bruinsma, 2012) while agriculture environmental footprint must decrease dramatically (Foley et al., 2011). In developing countries, agriculture production must increase 80% through higher yields resulting from intensification of existing agricultural systems (Conforti, 2011). Sustainable intensification was defined as a form of production wherein yields are increased without adverse environmental impact and without the cultivation of more land (Royal Society, 2009). Despite contested (Struik and Kuyper, 2017), this term was deeply discussed (Pretty and Bharucha, 2014) and highlights the needs to increase the productivity (i.e. agricultural product outputs per hectare) of current agricultural systems through practices that minimize key environmental issues (Garnett and Godfray, 2012).

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[1] Congio, G.F.S., Batalha, C.D.A., Chiavegato, M.B., Berndt, A., Oliveira, P.P.A., Frighetto, R.T.S., Maxwell, T.M.R., Gregorini, P., Da Silva, S.C., 2018. Strategic grazing management towards sustainable intensification at tropical pasture-based dairy systems. Sci. Total Environ. 636:872–880. DOI: 10.1016/j.scitotenv.2018.04.301

Intensification of pasture-based dairy systems has been associated with increasing inputs such as nitrogen fertilizer or imported supplements (Beukes et al., 2012; Foote et al., 2015; Macdonald et al., 2017). However, such intensification practices are associated with issues of environmental concern, namely increased greenhouse gases (GHG) emissions, water and land degradation (Foley et al., 2011; Vogeler et al., 2013; Foote et al., 2015). Alternatively, grazing management strategies that optimize herbage utilization and digestible dry matter intake (DMI) by grazing cows could improve land-use and mitigate key environmental issues of pasture-based dairy systems (Muñoz et al., 2016; Gregorini et al., 2017).

Plant growth is a function of canopy light interception (LI) and leaf area index (LAI), with the accumulation of herbage fitted to a sigmoid curve with three distinct phases (Brougham, 1955). During the early stages of regrowth, leaves are the morphological component accumulated the most. As LAI increases, canopy light intra-competition increases and plants change their growth pattern as a means of optimizing light capture through stem elongation. The shift in growth pattern occurs when canopy LI reaches and exceeds 95% ($LI_{95\%}$; Da Silva et al., 2015). Intermittent grazing practices (i.e. rotational stocking), interrupting regrowth at $LI_{95\%}$, leads to a greater leaf accumulation (Pereira et al., 2014; Pereira et al., 2015b), higher tiller population density and soil cover (Pereira et al., 2015a) than grazing at maximum light interception (LI_{Max}). In addition, sward grazed at $LI_{95\%}$ has been reported to have herbage of greater nutritive value (Trindade et al., 2007) and less herbage losses (Silveira et al., 2013).

Considering the grazing animal, pre-grazing management targets which optimize leaf production and nutritive value ($LI_{95\%}$) would maximize herbage DMI owing to the greater proportion of leaves in the grazing strata (Da Silva and Carvalho, 2005; Gregorini et al., 2011). Optimum short-term intake rate by dairy heifers grazing guinea grass was obtained when sward intercepted 95% of the incident light (Carnevalli et al., 2006; Palhano et al., 2007). Enteric methane (CH_4) is the predominant source of GHG emissions in livestock systems (Crosson et al., 2011; Guerci et al., 2013), ranging from 30% (high feed concentrate levels) to 83.5% (pasture-based) of total GHG emissions in dairy farming systems (Aguirre-Villegas et al., 2017). Enteric CH_4 production from animal digestion is associated with feed intake and herbage chemical composition (Janssen, 2010). In temperate grasslands, grazing strategies can be used to reduce the CH_4 emission intensity (i.e. CH_4/kg of product) and CH_4 yield (i.e. CH_4/kg of DMI) (Wims et al., 2010; Boland et al., 2013; Muñoz et al., 2016).

Although the studies aforementioned have demonstrated the benefits of grazing strategies based on $LI_{95\%}$ criteria, most focused solely on plant responses. There is a knowledge gap in relationships among plant and animal responses and environmental benefits in tropical pasture-based dairy systems. The central hypothesis of this study is that the change in sward structure caused by $LI_{95\%}$ management would optimize processes related to plant growth, plant-animal interface and between animal-rumen microorganisms delivering improved environmental services to the system by reducing CH_4 emission intensity and increased milk productivity. Our objective was to investigate the influence of strategic grazing with pre-grazing targets ($LI_{95\%}$ and LI_{Max}) on enteric CH_4 emissions and animal productivity in dairy tropical pasture of elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon).

3.2. Material and Methods

All procedures for this study were approved by the Animal (15.5.1246.11.2) and Environment Ethics Committees (17.5.999.11.9) at the University of São Paulo, College of Agriculture “Luiz de Queiroz” (USP/ESALQ).

3.2.1. Study site

The experiment was conducted in Piracicaba, SP, Brazil (22°42′S, 47°38′W and 546 a.s.l.) on a rainfed, non-irrigated elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon) pasture established in 1972 in a high fertility Eutroferric Red Nitossol (Pereira et al., 2014). The climate is sub-tropical with dry winters and 1328 mm average annual rainfall (CEPAGRI, 2012). The lowest and highest mean temperatures were recorded in July (19.7 °C) and December (27.1 °C), respectively. The greatest accumulated rainfall was observed from late spring to summer (1090 mm from November 2015 to March 2016), and the lowest from winter to early spring (356 mm from June to October 2015).

3.2.2. Treatments and experimental design

The two treatments were pre-grazing targets of either 95% or maximum canopy light interception during regrowth ($LI_{95\%}$ and LI_{Max}, respectively). Treatments were allocated to experimental units (2058 m^2 paddocks) according to a randomized complete block design, with six replications. The slope and chemical soil characteristics were considered as blocking criteria.

Before treatment implementation, paddocks were grazed and mowed to 45 cm for standardization in mid-January 2015. The pre-grazing targets of $LI_{95\%}$ and LI_{Max} were maintained until late November 2015 (adaptation period). This period was necessary to adapt sward structure to treatments and to identify the pre-grazing sward surface height (SSH) for the pre-grazing targets ($LI_{95\%}$ and LI_{Max}). For both treatments, the herbage depletion level (HDL) corresponded to 50% of the pre-grazing SSH as a means to maintain high short-term rates of herbage intake (Fonseca et al., 2012; Carvalho, 2013). The pre- and post-grazing SSH were measured from ground level to the top leafy horizon by 40 systematic readings per paddock, using a stick graduated in centimeters. Canopy LI was monitored using a LAI 2000 canopy analyzer (LI-COR, Lincoln, NE, USA) to take six readings above the canopy and thirty at ground level per experimental unit (Pereira et al., 2014).

Measurements were performed after the adaptation period throughout the experimental period (from December 4th 2015 to April 3th 2016 – 119 days), which was divided into three sampling periods of forty days (early summer, full summer and late summer). During the experimental period, pre-grazing targets for grazing management treatments were based on the heights corresponding to the LI treatments determined during the adaptation period. A total of 215 kg N/ha (as urea, 45% of N) was applied throughout the experimental period. Because the grazing interval was not constant (as a consequence of experimental treatments design), the total amount of N to be applied was divided throughout the experimental period (119 days) and a daily rate of nitrogen fertilization was calculated. The amount of N applied per paddock after each grazing was proportional to the length of the corresponding rest period (daily rate × rest period), ensuring similar N fertilization to both treatments at the end of the experimental period (Da Silva et al., 2017).

3.2.3. Plant measurements

Frequency of tussocks, bare ground, and weeds as well as tussock perimeter were measured five times throughout the adaptation and experimental periods. At the beginning of a regrowth cycle, a nylon string transect was placed within the paddock, with readings taken to identify botanical composition every two meters. Tussocks present at each point had their perimeter measured at ground level using a metric tape. A total of 100-points were sampled per paddock and the frequency of each botanical component was calculated as a proportion of the total number of reading points (Pereira et al., 2015a). At the last evaluation of botanical composition, tiller population density was determined by counting the total number of tillers in three rectangular sub-samples (0.94 m^2 each) randomized per paddock.

At the beginning of the experimental period, each paddock was divided up into three sub-paddocks (686 m^2) with plant measurements performed within the central sub-paddock. The SSH was measured as described above with 40 readings per sub-paddock. Pre-grazing herbage mass was quantified in each grazing cycle from three rectangular samples collected randomly (0.94 m^2 each) from each sub-paddock. The herbage was clipped above post-grazing SSH according to each treatment, weighed fresh, and two sub-samples taken to the laboratory. One sub-sample was used to determine plant-part components by hand separation into leaf (leaf blades), stem (stems + leaf sheaths) and dead material. The second sub-sample was used to determine herbage chemical composition. Both samples were dried in a forced-air drier at 65 °C to constant weight. Herbage and morphological components accumulation represent the sum of pre-grazing herbage mass throughout the experimental period. Samples to determine herbage chemical composition were ground through a 1-mm screen

(Wiley Mill, Thomas Scientific, Philadelphia, PA). Dry matter (DM) and ash concentrations were determined at 105 °C for 24 h and 600 °C for 4 h, respectively (AOAC International, 2005). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin concentrations were determined sequentially (Van Soest et al., 1991). Total nitrogen (N) concentration was determined by the Dumas combustion method using N analyzer (Leco FP-2000 N Analyzer; Leco Instruments Inc., St. Joseph, MI, USA), and crude protein (CP) concentration calculated as N × 6.25.

Grazing losses were estimated from two randomized samples (0.94 m^2 each) per paddock. At pre-grazing, rectangular frames were placed on the soil surface and all litter removed leaving a clean soil surface. After grazing, these areas were revisited and all material lying on the ground as well as broken stems and green leaves still attached and hanging on tussocks were collected, weighed fresh, and dried in a forced-air drier at 65 °C to constant weight (Silveira et al., 2013). Grazing losses were expressed in DM/ha and as a percentage of the pre-grazing herbage mass (above post-grazing SSH) and its complement to 100 was considered as grazing efficiency (Carnevalli et al., 2006). Herbage and leaf allowance were calculated by the relationship between pre-grazing herbage mass (above post-grazing SSH) and number of cows per day (Pérez-Prieto and Delagarde, 2013).

3.2.4. Herd and feeding

Twenty-six Holstein × Jersey dairy cows averaging 488 ± 60 kg body weight (BW) (mean ± SD), 2.94 ± 0.18 body condition score (BCS), daily milk yield of 20.3 ± 2.6 kg/d, and 126 ± 90 days in milk (DIM) were stratified and grouped in pairs into 13 blocks according to daily milk yield and DIM, and then randomly assigned to either $LI_{95\%}$ and LI_{Max} grazing management. An additional herd of dry-cows (10 to 13 cows) was maintained in an adjacent area of elephant grass and was used to keep grazing management targets constant, as needed. The stocking rate was calculated by number of cows used daily for each treatment, considering experimental cows and the additional herd.

Concentrate meals were fed individually twice daily (4:30 am and 2:30 pm) before milking (5 am and 3 pm) at a rate of 1 kg of concentrate/3 kg of milk (considering the average of each block). The rate was established based on milk yield at the beginning of each sampling period (Danes et al., 2013). The concentrate meal was composed of citrus pulp (35%), corn gluten feed (30%), fine ground corn (20%), soybean meal (10%) and mineral (5%), with chemical composition as followed: 88.4% DM, 10.3% ash, 14.0% CP, 22.2% NDF, 9.3% ADF, 3.3% ether extract and 49.8% non-fibrous carbohydrate.

3.2.5. Animal measurements

The BW and BCS were measured at the end of each sampling period over three consecutive days (Edmonson et al., 1989). Milk yield was recorded daily with samples collected in vials containing bronopol preservative pill and analyzed for fat, protein, lactose, and milk solids using infrared procedures (MilkoScan FT+; Foss North America Inc., Eden Prairie, MN).

Herbage intake was estimated from total fecal excretion and feed indigestibility. To estimate total fecal excretion, titanium dioxide (TiO_2) was dosed twice a day (20 g/cow per day) after concentrate meals over 12 days. Fecal samples were collected from the rectum after concentrate meals on the last 5 days, dried in a forced-air drier at 55 °C for 72 h, ground through a 1-mm screen (WileyMill, Thomas Scientific, Philadelphia, PA) and composited forming one sample per sampling period by cow. Titanium dioxide concentration in feces was determined according to Myers et al. (2004). To determine the feed indigestibility, the indigestible NDF (iNDF) content of herbage, concentrate, and fecal samples were estimated by 240 h *in vitro* incubation (Goeser and Combs, 2009). Total fecal excretion, fecal excretion from concentrate, and herbage intake were calculated according to De Souza et al. (2015).

Enteric CH_4 emissions were estimated using sulfur hexafluoride (SF_6) as tracer gas (Johnson and Johnson, 1995). Pre-calibrated permeation tubes containing SF_6 with known release rates (1.41 ± 0.40 mg/day) were placed into the rumen of each cow 72 h prior to the first collection. Sampling apparatus included a PVC collection canister (2.3 L) and adjustable halter containing stainless steel capillary tubing and brass connections. The cows were adapted to the sampling apparatus over 7 days prior to collection with CH_4 emissions measured at 24-hour intervals over 7 consecutive days. Canisters were vacuumed to approximately −13.5 psi using a three-stage vacuum pump (Symbol, Sumaré, SP, Brazil) and Druck DPI 705 digital manometer (GE Druck, South Burlington, VT, EUA) and replaced daily just after the afternoon concentrate meal. Background SF_6 and CH_4 concentrations were determined using two sampling apparatus placed daily in the field near the grazing herd. Methane and SF_6 concentrations were determined at the Laboratory of Biogeochemistry and Tracer Gases Analysis (Embrapa Meio Ambiente, Jaguariúna, SP, BRA) using gas chromatography (HP6890, Agilent, Delaware, USA). Prior to chromatograph determination, canisters were pressurized to 1.3–1.5 psi with ultrapure nitrogen 5.0, and pressures recorded by Druck DPI 705 digital manometer (GE Druck, South Burlington, VT, EUA) in order to calculate the dilution factor. The chromatograph was equipped with a flame ionization detector (FID) at 280 °C for CH_4 (column megabore, 0.53 mm × 30 m × 15 µm, Plot HP-Al/M) and an electron capture detector (ECD) at 300 °C for SF_6 (column megabore, 0.53 mm × 30 m × 25 µm, HP-MolSiv), with two loops of 0.5 cm^3 maintained at 80 °C attached to 2 six-way valves. Calibration curves were established using standard certified gases for CH_4 (4.85 ± 5%; 9.96 ± 1.65% and 19.1 ± 3.44% ppm) and SF_6 (34.0 ± 9.0; 91.0 ± 9.0 and 978.0 ± 98.0 ppt) (Westberg et al., 1998). Daily methane emission was calculated from collected SF_6 and CH_4

concentrations in the canisters discounting background concentrations, and the value of SF_6 permeation tube release rate (Johnson and Johnson, 1995).

3.2.6. Statistical analysis

Analysis of variance was performed using the Mixed Procedure (SAS 9.3; SAS Institute Inc., Cary, NC). Different structures of the variance-covariance matrices were tested and the Bayesian Information Criterion was adopted to select the best fit matrix. Within plant parameters, the paddock was considered the experimental unit, and for animal measurements, the cow was considered the experimental unit. Blocks were considered random terms, and LI, sampling periods and their interactions were treated as fixed effects. Sampling periods were treated as repeated measures. For tussock measurements, season of the year was considered a fixed effect because assessments were made throughout the entire study period (adaptation + experimental). Means were calculated using the LSMEANS statement, compared using the Student's t-test and the Bonferroni adjustment. Differences were declared significant at $P \leq 0.05$, and trends were declared at $P \leq 0.10$.

3.3. Results

3.3.1. Canopy light interception and sward surface height

Grazing management targets and sward characteristics during the adaptation and experimental periods are presented in Table 1. The $LI_{95\%}$ pre-grazing target was reached at 99 cm (≈100 cm) and the LI_{Max} pre-grazing target was reached at 134.5 cm (≈135 cm). For both treatments, HDL was close to the target of 50% of the pre-grazing SSH, and corresponded to post-grazing heights of 50.4 and 64.3 cm for $LI_{95\%}$ and LI_{Max}, respectively.

Table 1. Canopy light interception, pre- and post- grazing sward surface height (SSH) and herbage depletion level (HDL) of elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}) during the adaptation (Jan-Nov 2015) and experimental (Dec 2015-Apr 2016) periods (n = 6)

Period	**Treatments**		**SEM**[1]	***P*-value**
Adaptation	**$LI_{95\%}$**	**LI_{Max}**		
Light interception, %	95.2	98.0	0.11	<0.0001
Pre-SSH, cm	99.0	134.5	0.94	<0.0001
Post-SSH, cm	50.4	64.3	0.86	<0.0001
HDL, % of Pre-SSH	49.0	50.8	0.57	0.03
Experimental				
Pre-SSH, cm	99.7	134.4	0.58	<0.0001
Post-SSH, cm	50.9	68.3	0.45	<0.0001
HDL, % of Pre-SSH	49.1	48.9	0.46	0.7269

[1] Standard error of the mean

3.3.2. Canopy cover

Frequencies of tussocks and bare ground, and tussock perimeter were affected by season ($P < 0.01$) indicating a strong effect of growth conditions on plant ecophysiology (Table 2). Across seasons of the year, tussocks showed a tendency of greater frequency ($P = 0.06$) for $LI_{95\%}$ than LI_{Max} (38% and 33%, respectively). Inversely, the frequency of bare ground was greater ($P = 0.04$) for LI_{Max} than $LI_{95\%}$ (54% and 50%, respectively). There was no effect of LI pre-grazing on tussock perimeter. However, in the second summer, tussocks under $LI_{95\%}$ management had a greater perimeter than LI_{Max} ($P < 0.05$). Tiller population density was greater ($P < 0.01$) for $LI_{95\%}$ relative to LI_{Max}.

Table 2. Frequencies of tussock and bare ground, tussock perimeter and tiller population density of elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}) during the adaptation (Jan-Nov 2015) and experimental (Dec 2015-Apr 2016) periods (n = 6)

	Seasons[1]					**SEM**[2]	***P*-value**		
	S1	**A/W**	**ES**	**LS**	**S2**		**Trt**[3]	**Per**[4]	**Trt×Per**
	Frequency of tussocks, %								
$LI_{95\%}$	32.8 Ab	33.7 Ab	28.6 Ab	35.7 Ab	52.9 Aa	3.30	0.0633	<0.0001	0.6955
LI_{Max}	32.9 Ab	29.9 Ab	27.5 Ab	26.8 Ab	47.7 Aa				
	Frequency of bare ground, %								
$LI_{95\%}$	54.0 Aa	54.9 Aa	59.7 Aa	51.8 Aa	28.8 Ab	3.23	0.041	<0.0001	0.8760
LI_{Max}	56.2 Aa	57.4 Aa	62.2 Aa	60.5 Aa	32.4 Ab				
	Tussock perimeter, cm								
$LI_{95\%}$	174 Ab	186 Ab	193 Ab	165 Ab	236 Aa	9.15	0.795	<0.0001	0.0604
LI_{Max}	185 Aa	186 Aa	183 Aa	187 Aa	205 Ba				
	Tiller population density, tiller/m^2								
$LI_{95\%}$	-	-	-	-	129.1	6.53	0.0049	-	-
LI_{Max}	-	-	-	-	87.3				

Means followed by the same capital letter in columns and the lower case letter in rows do not differ ($P > 0.05$)

[1] S1: Summer 1 – Mar 2015, A/W: Autumn/Winter – Jun/Jul 2015, ES: Early Spring – Sep 2015, LS: Late Spring – Nov 2015 and S2: Summer 2 – Dec 2015

[2] Standard error of the mean

[3] Treatment effect

[4] Sampling period effect

3.3.3. Herbage characteristics

The $LI_{95\%}$ provided more grazing cycles (*P* < 0.01) associated with lower stocking and shorter rest periods than LI_{Max} (*P* < 0.01) (Table 3). Longer rest periods of LI_{Max} allowed greater plant growth and determined higher pre-grazing herbage mass (*P* < 0.01) with more stems (*P* < 0.01), lower leaf blade (*P* < 0.01) and lower leaf:stem ratio (*P* < 0.01) than $LI_{95\%}$. There was no treatment effect on dead material (*P* = 0.31). Herbage accumulation was not affected by LI strategy (*P* = 0.11) but more frequent defoliation ($LI_{95\%}$) resulted in greater leaf (*P* = 0.01) and lower stem (*P* < 0.01) accumulation throughout the experimental period (Table 3). Daily herbage allowances were greater for LI_{Max} than $LI_{95\%}$ (*P* = 0.02). The $LI_{95\%}$ promoted lower grazing losses (*P* < 0.01) and more efficient grazing (*P* < 0.01) than LI_{Max}. Grazing management strategy influenced herbage chemical composition, with $LI_{95\%}$ herbage having greater CP (*P* < 0.01), and lower ADF (*P* = 0.03) and lower lignin (*P* < 0.01; Table 3). There was no treatment effect on DM (*P* = 0.70), NDF (*P* = 0.11), and ash (*P* = 0.28) (Table 3).

Table 3. Grazing cycles, stocking period, rest period, pre-grazing herbage characteristics, herbage accumulation, herbage allowance, grazing losses, grazing efficiency and herbage chemical composition of elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}) during the experimental period (Dec 2015-Apr 2016) (n = 6)

Item	**Treatments**		**SEM**[1]	***P*-value**		
	$LI_{95\%}$	**LI_{Max}**		**Trt**[2]	**Per**[3]	**Trt×Per**
Grazing cycles, n	5.6	3.5	0.16	<0.0001	0.1330	0.2430
Stocking period, days	1.0	1.4	0.06	<0.0001	0.0582	0.0917
Rest period, days	21.1	31.7	0.60	<0.0001	<0.0001	0.5215
Pre-grazing herbage mass[4], kg of DM/ha	2890	4890	207.9	<0.0001	0.3625	0.3410
Leaf blade[4], %	95.1	81.1	0.93	<0.0001	0.7648	0.1049
Stem[4], %	3.5	16.6	0.52	<0.0001	0.0919	0.2000
Dead material[4], %	1.4	2.3	0.20	0.3132	0.0137	0.4464
Leaf : Stem ratio[4]	32.8	4.6	3.50	<0.0001	0.0011	0.0004
Herbage accumulation[4], kg of DM/ha	15441	16683	847.0	0.1079	-	-
Leaf accumulation[4], kg of DM/ha	14611	13276	730.2	0.0134	-	-
Stem accumulation[4], kg of DM/ha	795	3322	264.2	<0.0001	-	-
Herbage allowance[4], kg of DM/cow.day	21.2	29.7	2.53	0.016	0.5026	0.4409
Grazing losses, kg of DM/ha	292	1203	100.3	<0.0001	<0.0001	<0.0001
Grazing efficiency, %	89.3	79.9	2.88	0.0003	0.004	0.7755
Herbage chemical composition, % DM						
Dry matter	19.5	19.2	0.91	0.6992	0.0724	0.2753
Crude protein	21.0	19.4	0.50	0.0004	<0.0001	0.0387
Neutral detergent fiber	61.2	63.0	1.26	0.1121	0.1438	0.2865
Acid detergent fiber	33.9	36.3	1.14	0.0248	0.2603	0.7348
Lignin	3.3	3.8	0.16	0.0040	0.2429	0.4676
Ash	10.4	11.2	0.54	0.2816	0.0228	0.6754

[1] Standard error of the mean

[2] Treatment effect

[3] Sampling period effect

[4] Estimated above post-grazing SSH

3.3.4. Dry matter intake, animal performance and CH_4 emissions

Animal responses are presented in Table 4. Stocking rate was 33% greater for $LI_{95\%}$ than LI_{Max} ($P < 0.01$). Greater herbage ($P < 0.01$) and total DMI ($P < 0.01$) were observed for $LI_{95\%}$ than LI_{Max}. Grazing at $LI_{95\%}$ resulted in 15.3% greater milk yield ($P < 0.01$), 8.9% more fat corrected milk ($P = 0.02$), 8% greater protein ($P < 0.01$), 15.3% more lactose ($P < 0.01$) and 6.2% greater milk solids ($P < 0.01$) yields. Fat yield was not affected by treatment ($P = 0.20$). There were no LI effects on BW and BCS changes ($P = 0.61$ and $P = 0.13$, respectively). Daily enteric CH_4 emission (g/d) was not affected by treatment ($P = 0.85$), however $LI_{95\%}$ grazing management increased the efficiency of milk ($P < 0.01$), fat ($P < 0.01$), protein ($P < 0.01$) and milk solids ($P < 0.01$) yields per g of CH_4 emitted by 21%, 15%, 13% and 16%, respectively. Additionally, cows grazing elephant grass managed with the $LI_{95\%}$ pre-grazing target had lower CH_4 yield ($P = 0.02$).

Table 4. Stocking rate, daily dry matter intake (DMI), milk yield and enteric CH_4 emissions of cows grazing elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}) during the experimental period (Dec 2015-Apr 2016) (n = 13)

Item	**Treatments**		**SEM**[1]	***P*-value**		
	$LI_{95\%}$	**LI_{Max}**		**Trt**[2]	**Per**[3]	**Trt×Per**
Stocking rate, cows/ha	9.3	7.0	0.98	0.0054	-	-
Daily DMI, kg of DM/cow						
Herbage	12.3	10.1	0.52	0.0017	0.0071	0.1781
Total	18.2	15.9	0.61	0.0028	0.1113	0.2177
Yield, kg/d						
Milk	18.1	15.7	1.01	<0.0001	<0.0001	0.7768
3.5% FCM[4]	18.4	16.9	1.18	0.0205	0.0156	0.6329
Fat	0.646	0.608	0.0407	0.2004	0.0709	0.355
Protein	0.556	0.515	0.0274	0.0098	0.001	0.8361
Lactose	0.792	0.687	0.0461	<0.0001	<0.0001	0.5586
Milk solids	2.07	1.95	0.1084	0.0059	0.0004	0.7953
BW[5] change, kg/d	0.4375	0.5530	0.36	0.6107	0.0031	0.6086
BCS[6] change	-0.06	0.01	0.05	0.1330	0.8276	0.7137
CH_4 emissions						
g/d	297.8	296.1	13.30	0.8533	<0.0001	0.8978
g/kg of milk yield	16.2	20.5	1.09	<0.0001	<0.0001	0.1365
g/kg of fat yield	438.9	515.3	24.71	0.0005	<0.0001	0.9986
g/kg of protein yield	525.2	604.6	22.40	<0.0001	<0.0001	0.1931
g/kg of milk solids yield	133.5	159.5	6.71	<0.0001	<0.0001	0.4666
g/kg of dry matter intake	20.2	24.7	1.33	0.0199	<0.0001	0.0012

[1] Standard error of the mean

[2] Treatment effect

[3] Sampling period effect

[4] 3.5% Fat Corrected Milk = [(0.4324 × milk yield) + (16.216 × fat yield)]

[5] BW: body weight

[6] BCS: body condition score

3.3.5. Milk yield and CH_4 emissions per hectare

Milk yield and CH_4 emissions per hectare are presented in Table 5. Grazing at $LI_{95\%}$ increased milk yield by 51% ($P < 0.01$), fat yield by 42% ($P = 0.02$), protein yield by 41% ($P < 0.01$) and milk solids yield by 40% ($P < 0.01$) per hectare. The enteric CH_4 emitted per hectare was 29% greater for $LI_{95\%}$ than LI_{Max} ($P < 0.01$). Additionally, $LI_{95\%}$ pre-grazing target increased productivity of milk ($P < 0.01$), protein ($P < 0.01$) and milk solids ($P < 0.01$) per kg of CH_4 released per hectare by 16%, 9% and 7%, respectively (Table 5).

Table 5. Milk yield and CH_4 emissions per hectare (ha) of cows grazing elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}) during the experimental period (Dec 2015-Apr 2016) (n = 6)

Item	**Treatments**		**SEM**^{1}	***P*-value**
	$LI_{95\%}$	**LI_{Max}**		
Milk, kg/ha.day	169.8	112.4	20.40	0.0012
Fat, kg/ha.day	6.1	4.3	0.71	0.0148
Protein, kg/ha.day	5.2	3.7	0.62	0.0026
Milk solids, kg/ha.day	19.5	13.9	2.42	0.0057
CH_4, kg/ha per day	2.7	2.1	0.24	0.0055
Productivity vs. CH_4				
Milk, kg/kg of CH_4/ha.day	62.2	53.8	4.79	0.0002
Fat, kg/kg of CH_4/ha.day	2.22	2.06	0.132	0.1151
Protein, kg/kg of CH_4/ha.day	1.91	1.76	0.135	<0.0001
Milk solids, kg/kg of CH_4/ha.day	7.13	6.66	0.550	0.0098

^{1}Standard error of the mean

3.4. Discussion

Grazing strategies that maintain shorter rather than taller pre-grazing SSH normally result in greater canopy cover, associated with greater frequency of tussocks and tiller population density (Pereira et al., 2015a). Greater canopy cover by plants reduces nutrient and soil transport avoiding contamination and sedimentation of waterways (McDowell and Houlbrooke, 2009). Furthermore, competition for light is stronger in taller swards, resulting in tiller death, reduced tillering, less stability of plant population, greater frequency of bare ground and reduced sward perennation (Pereira et al., 2015a). The differences observed in tussock perimeter during the second summer, one year after the adaptation period, indicate that elephant grass adapts its horizontal structure to defoliation regimes slowly. This feature is an important aspect to be considered for planning of field experiments and management strategies (Pereira et al., 2015a).

The $LI_{95\%}$ pre-grazing target results in maximum net herbage accumulation rate and is considered the critical LAI (Brougham, 1958). For tropical forage, this condition is associated with the beginning of marked stem elongation (Da Silva et al., 2015). Pre-grazing SSH is strongly correlated with LAI and LI, and can be used as a reliable field indicator for controlling herbage regrowth.

Grazing strategies based on the maximum LI pre-grazing target result in longer regrowth periods, increasing light competition within the sward, shifting the plant's growth pattern to prioritize stem elongation and resulting in greater stem and lower leaf accumulation (Da Silva et al., 2009). In the present study, the 3-percentual unit increase in LI pre-grazing for the LI_{Max} relative to the $LI_{95\%}$ target resulted in 50% increase of the rest period. Total herbage accumulation was similar between treatments. However, $LI_{95\%}$ increased leaf accumulation by 10% and decreased stem accumulation by 76% compared to LI_{Max}, as previously shown (Carnevalli et al., 2006; Barbosa et al., 2007; Silveira et al., 2013; Pereira et al., 2014).

In pasture-based systems, only a portion of the herbage accumulated is consumed by grazing animals. The remaining fraction is lost as a consequence of trampling and characterizes grazing inefficiency (Carnevalli et al., 2006; Silveira et al., 2013). Grazing at LI_{Max} resulted in fourfold higher grazing losses than $LI_{95\%}$. This suggests that taller swards result in greater grazing losses, as observed by previous studies in tropical conditions (Carnevalli et al., 2006; Silveira et al., 2013). Inversely, the grazing efficiency was ten percentage units higher for $LI_{95\%}$ than LI_{Max}, corroborating the findings of Carnevalli et al. (2006) and Silveira et al. (2013).

The stocking rate was 33% greater for $LI_{95\%}$ than LI_{Max}. Voltolini et al. (2010) and Gimenes et al. (2011) found stocking rate increases ranging from 10 to 42% in elephant grass and palisade grass pastures adopting the 95% pre-grazing target of canopy light interception under rotational grazing management. Greater stocking rates are supported by greater leaf accumulation associated with lower grazing losses determining greater grazing efficiency. In the present study, $LI_{95\%}$ had 10% greater leaf accumulation associated with fourfold reduction in grazing losses. The lower grazing efficiency observed in taller swards may also be associated with higher stem accumulation and increased senescence and dead material (Pereira et al., 2014; Pereira et al., 2015b), which are plant-part components avoided by grazers (Trindade et al., 2007).

Chemical composition of the herbage is a function of the proportions of plant-part components and their tissue anatomy (Moore, 1994). In the present study, stem proportions were 3.5% for $LI_{95\%}$ and 16.6% for LI_{Max} and, leaf blade was 95.1% for $LI_{95\%}$ and 81.1% for LI_{Max}. The ADF is composed of cellulose and lignin, present mainly in the cell wall, associated with structural support for plant organs (Moore and Jung, 2001). Stems contain a higher proportion of cell wall tissues and less photosynthetic tissues than leaves (Wilson and Kennedy, 1996). On the other hand, most protein compounds are present in leaves, with the majority associated with photosynthetic enzymes (Gastal and Durand, 2000).

Daily herbage intake is determined by interactions between sward structure and grazing animals (Wade and Carvalho, 2000). Poppi et al. (1987) suggested that herbage intake by grazing animals follows an asymptotic distribution represented by two distinct phases. In the first ascending phase, herbage intake is related to sward structure (herbage or leaf mass, pre-SSH, leaf-to-stem ratio) and grazing behavior (grazing time, diet selection, bite mass and bite rate), which are characteristics strongly affected by grazing management strategies (Da Silva and Carvalho, 2005). In the second asymptotic phase, nutritional factors such as herbage chemical composition, time of herbage retention in the rumen and concentration of metabolic compounds are more relevant in controlling intake (Poppi et al., 1987). In the present study, it is likely that sward structure characteristics, such as pre-grazing SSH and plant-part components strongly affected grazing behavior and ultimately herbage DMI. Swards constantly kept at taller heights (such as LI_{Max} swards) result in lower short-term intake rate at the beginning of grazing, due to the excessive length of leaf blade associated with lower bulk density of herbage in the upper strata which increases time per bite (Palhano et al., 2007; Fonseca et al., 2013; Carvalho, 2013). At the end of grazing, greater proportion of stems results in physical constraints reducing herbage intake (Laca and Lemaire, 2000; Fonseca et al., 2012; Carvalho, 2013). At the rumen level, more fibrous herbage (i.e. higher NDF, ADF and lignin) is associated with greater ruminal retention time, lower fermentation and passage rate, and lower herbage intake (Mertens, 1994; Allen, 1996; Allen, 2000; Forbes, 2007).

Herbage DMI is a key determinant of performance of livestock-based systems (Mertens, 1994; Poppi et al., 1997; Sollenberger and Burns, 2001; Coleman and Moore, 2003; Sollenberger and Vanzant, 2011). Coleman and Moore (2003) described that the combination of herbage chemical composition (i.e. nutritive value), nutrient availability (i.e. digestibility), and intake determines herbage quality (i.e. feed value) and is accepted as an indicator of animal performance. Our results indicate that it is possible to produce herbage with high nutritive value resulting in greater herbage DMI, and milk yield through pre-grazing SSH that avoids excessive stem elongation ($LI_{95\%}$). These results corroborate those of experiments conducted in temperate climates that determined greater nutritive value, DMI and milk yield in low herbage mass swards compared to high herbage mass swards (Wims et al., 2010; Muñoz et al., 2016). In the present study, greater milk yield was observed at higher stocking rates and lower herbage allowance ($LI_{95\%}$) demonstrating that in tropical swards, the distribution and arrangement of above-ground plant parts (i.e. sward structure, see Laca and Lemaire, 2000) plays a more important role than herbage allowance in determining herbage DMI and animal performance.

Enteric CH_4 is affected by the amount and nature of feed, and the extent of its degradation, which in turn determines the amount of hydrogen formed in the rumen (Janssen, 2010). Although DMI and herbage nutritive value have been affected by targets of pre-grazing LI, daily enteric CH_4 emission was not. Similarly, studies with temperate grasses did not observe differences in daily enteric CH_4 emission from beef heifers (Boland et al., 2013) and dairy cows (Wims et al., 2010; Muñoz et al., 2016) grazing low herbage mass and high herbage mass swards, even with significant differences reported in DMI and nutritive value. The model proposed by Janssen (2010) suggests that greater passage rates increase hydrogen concentration in the rumen. Consequently, microorganisms would select pathways thermodynamically more favorable to this condition, which produce less hydrogen resulting in less CH_4 formed per unit of feed ingested. It is possible that for the DMI ranges observed in the present study, reductions in CH_4 yield (g/kg of DMI) have compensated the greater DMI, decreasing daily CH_4 emissions. Our results indicate reductions around 20% for emission intensity (g/kg of milk yield) and CH_4 yield when adequate grazing management was adopted ($LI_{95\%}$ pre-grazing target), while Wims et al. (2010) and Muñoz et al. (2016) reported reductions of 10% for temperate swards. It is worth mentioning that CH_4 emission intensity of $LI_{95\%}$ was 16.8 g of CH_4/kg of milk yield, lower than results reported in temperate pastures (18.8 g of CH_4/kg of milk yield), and the methane yield of $LI_{95\%}$ was similar to results obtained on temperature pastures (20.2 vs. 21.5 g of CH_4/kg of DMI, values from Wims et al., 2010; Enriquez-Hidalgo et al., 2014; Muñoz et al., 2016). These results highlight the need to review the historical concept of tropical grasses having low herbage quality when managed under tight sward monitoring (Stobbs, 1975; Sollenberger and Burns, 2001).

Milk yield outputs per hectare increased between 40 and 50% by simply changing the pre-grazing SSH of elephant grass from ≈135 cm to 100 cm. Greater milk productivity was achieved by increased stocking rate (+33%) and milk yield per cow (+15%) when $LI_{95\%}$ was adopted. Greater milk yields in grazing dairy farms have been usually associated with the provision of additional feed (i.e. increased nitrogen rates onto pastures, external supplementary feed inputs; Ramsbottom et al., 2015; Macdonald et al., 2017). However, in addition to economic investments, both techniques are associated with environmental issues, such as increased GHG emissions, water and land degradation (Foley et al., 2011; Vogeler et al., 2013; Foote et al., 2015). Enteric CH_4 emitted per hectare was greater for lower pre-grazing SSH and it was a function of the higher stocking rates resulting from this grazing strategy. However, this result has a small relative importance when milk yield per hectare is considered. The 51% increase in milk productivity overcame the 29% increase in enteric CH_4 emissions per hectare for the $LI_{95\%}$ grazing management, and thereby determined a 16% mitigation of the main greenhouse gas from pasture-based dairy systems (Crosson et al., 2011; Guerci et al., 2013; Aguirre-Villegas et al., 2017).

In a context where the growing demand for food must be achieved through low environmental footprint practices, our findings highlighted an opportunity to improve the efficiency of tropical pasture-based dairy systems through optimization of ecological processes. Strategic grazing allows for intensification that is not coupled with increase in external resources (i.e. fertilizer, external supplements) but rather to efficient use of existing resources (i.e. solar radiation, rainwater, pasture, fertilizer, supplement). In addition, strategic grazing management is a non cost and readily available practice with easy adoption that enhances profitability of tropical pasture-based systems. The adoption of strategic grazing in tropical pasture-based systems provides the opportunity to either increase farms' total production, or use the extra land for food or forestry (further mitigating GHG emissions). The decision regarding land use depends on governmental policy or market trends.

3.5. Conclusions

Strategic grazing management that reduces stem elongation in tropical forage grass swards optimizes the processes inherent to plant growth (i.e. leaf accumulation and herbage nutritive value), to plant animal interface (i.e. grazing efficiency and DMI), and animal (i.e. CH_4 emission intensity and CH_4 yield), resulting in greater milk yield from the same area of land. Monitoring of SSH in tropical pastures is a useful and reliable field tool that translates ecophysiological plant responses in a practical manner to farmers towards sustainable intensification at pasture-based systems in the tropics.

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4. STRATEGIC GRAZING MANAGEMENT AND NITROUS OXIDE FLUXES FROM PASTURE SOILS IN TROPICAL DAIRY SYSTEMS

Abstract

Greenhouse gases emissions are considered the most important among all environmental issues of dairy farming systems. Nitrous oxide (N_2O) has particular importance owing to its global warming potential and stratospheric ozone depletion. The objective of this study was to investigate the influence of two rotational grazing strategies characterized by two pre-grazing targets (95% and maximum canopy light interception during sward regrowth; $LI_{95\%}$ and LI_{Max}, respectively) on N_2O fluxes from soil and milk productivity in a tropical dairy farming system based on elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon). The general hypothesis was that frequent defoliations generated by the $LI_{95\%}$ pre-grazing target would increase N_2O fluxes, however this greater emission would ultimately be compensated by greater milk productivity. Results indicated that $LI_{95\%}$ pre-grazing target provided more frequent defoliation than LI_{Max}. Water-filled pore space (WFPS), soil and chamber temperatures were affected by sampling period (P_1 and P_2). There was significant treatment × sampling period interaction on soil NH_4^+ concentration, and it was most likely associated with urinary-N discharge. During P_1, there was a greater urinary-N discharge for $LI_{95\%}$ than LI_{Max} (26.3 vs. 20.9 kg of urinary-N/paddock) caused by higher stocking rate (10.0 vs. 8.3 cows/ha), which resulted in greater N_2O fluxes for $LI_{95\%}$. Inversely, during P_2, the soil NH_4^+ and N_2O fluxes were greater for LI_{Max} than $LI_{95\%}$. During this period, the greater urinary-N discharge (46.8 vs. 44.8 kg of urinary-N/paddock) was likely associated with greater stocking period (1.88 vs. 1.46 days) for LI_{Max} relative to $LI_{95\%,}$ since both treatments had similar stocking rate (9.5 vs. 9.9 cows/ha). Converting hourly N_2O fluxes to daily basis and relating to milk productivity, $LI_{95\%}$ was 35% more efficient than LI_{Max} (0.36 vs. 0.55 g N-N_2O/kg milk.ha.day). In addition, $LI_{95\%}$ pre-grazing target decreased 34% urea-N applied per milk yield per hectare (0.57 vs. 0.86 g urea-N/kg milk.ha.day). Strategic grazing management represented by the $LI_{95\%}$ pre-grazing target allows for intensification of tropical pasture-based dairy systems enhancing milk productivity and decreasing N-N_2O emitted per kg of milk.

Keywords: Canopy light interception; Nitrous oxide fluxes; Grazed soils; Soil nitrogen; Land-use improvement; Elephant grass

4.1. Introduction

Greenhouse gases (GHG) emissions are considered the most important among all environmental issues of dairy farming systems (O’Brien et al., 2012; Guerci et al., 2013; Gregorini et al, 2016). Nitrous oxide (N_2O) has particular importance owing to its global warming potential (265-298 times greater than carbon dioxide; Myhre et al., 2013) and stratospheric ozone depletion (Ravishankara et al., 2009; IPCC, 2014). It is the main GHG from the soil and the second most representative among all GHG, ranging from 15 (housed) to 25% (pasture-based) of total GHG emissions in dairy farming systems (Aguirre-Villegas et al., 2017). Methane (CH_4) and carbon dioxide (CO_2) from soils are proportionally less important than N_2O in dairy farming systems (Jarvis et al., 1995; de Klein et al., 2008; Aguirre-Villegas et al., 2017).

Nitrous oxide is formed through microbial transformation of nitrogen (N) compounds into the soil, typically by incomplete denitrification or by nitrification (Wrage et al., 2001; Saggar et al., 2013). Nitrous oxide fluxes are affected by a wide range of proximal and distal regulators, making its regulation a very complex process (de Klein et al., 2008; Luo et al., 2017). Proximal soil factors include mineral nitrogen (NH_4^+ and NO_3^-) and organic carbon availabilities, moisture, pH, temperature, and texture that, in turn, are affected by distal regulators such as rainfall or irrigation, soil compaction, organic matter and N inputs (de Klein et al., 2008; Luo et al., 2017). Periods when soil characteristics favorable to N_2O production coincide are called “hot moments” (Luo et al., 2017). In tropical conditions, these “hot moments” usually occur during spring and summer when pastures are intensively growing owing to the abundance of solar radiation, rainfall, and N inputs.

Grazing management strategies can strongly affect the majority of distal regulators. It determines ecophysiological plant processes such as herbage growth, senescence and decay (Da Silva et al., 2009; Pereira et al., 2014; Pereira et al., 2015; Da Silva et al., 2015; Congio et al., 2018) that strongly affect animal responses such as herbage intake (Congio et al., 2018), herbage losses by cattle trampling (Carnevalli et al., 2006; Silveira et al., 2013; Congio et al., 2018), stocking rate (Voltolini et al., 2010; Gimenes et al., 2011; Congio et al., 2018), excreta spatial distribution (White et al., 2001; Auerswald et al., 2010), and N load into pastures (Vibart et al., 2017). These factors, in turn, modify soil properties (i.e. bulk density, moisture, temperature, pH, aeration) (Warren et al., 1986; Silva et al., 2003; Schmalz et al., 2013) that affect microbial community growth and activity (Bardgett et al., 1996; Bardgett et al., 2001; Bardgett and Wardle, 2003) determining the intensity of processes associated to N_2O fluxes from soils (de Klein et al., 2008; Levine et al., 2011; Luo et al., 2017).

The majority of studies involving N_2O flux from pasture soils have been addressed to assess the effects of proximal factors on processes and emission factors in temperate conditions (Saggar et al., 2013; de Klein et al., 2014; Barneze et al., 2015; Venterea et al., 2015; Gardiner et al., 2016; Samad et al., 2016; Clough et al., 2017; Gardiner et al., 2017; van der Weerden et al. 2017; Luo et al., 2018; Rex et al., 2018). The little information available for tropical pastures has also focused on nitrous oxide fluxes related to proximal factors within urine patches (Barneze et al., 2014; Lessa et al., 2014; Mazzetto et al., 2014; Mazzetto et al., 2015). There is no information available regarding N_2O fluxes from soils of tropical pasture-based dairy farming systems, as influenced by grazing management strategies. In fact, farming scale studies are scarce even in temperate conditions. Experimental approaches have shown that intensively managed grasslands are stronger sources of N_2O than extensively managed grasslands owing to greater inputs of N fertilizer and excreta (Smith et al., 2001; Flechard et al., 2007; Rafique et al., 2011). However, they have not accounted for animal outputs that are usually greater in intensively managed systems and could compensate the higher N_2O fluxes. The objective of this study was to investigate the influence of two rotational grazing strategies characterized by two pre-grazing targets (95% and maximum canopy light interception during sward regrowth; $LI_{95\%}$ and LI_{Max}, respectively) on N_2O fluxes from soil and milk productivity in a tropical dairy farming system based on elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon). The general hypothesis was that frequent defoliations generated by the $LI_{95\%}$ pre-grazing target would increase N_2O fluxes, however this greater emission would ultimately be compensated by greater milk productivity.

4.2. Material and Methods

All procedures for this study were approved by the Animal (15.5.1246.11.2) and Environment Ethics Committees (17.5.999.11.9) at the University of São Paulo, College of Agriculture "Luiz de Queiroz" (USP/ESALQ).

4.2.1. Study site

The experiment was conducted in Piracicaba, SP, Brazil (22°42′S, 47°38′W and 546 a.s.l.) on a rainfed, non-irrigated elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon) pasture established in 1972 in a high fertility Eutroferric Red Nitossol (Pereira et al., 2014). The climate is sub-tropical with dry winters and 1328 mm average annual rainfall (CEPAGRI, 2012). The lowest and highest mean temperatures were recorded in July (19.7 °C) and December (27.1 °C), respectively. The greatest accumulated rainfall was observed from late spring to summer (1090 mm from November 2015 to March 2016), and the lowest from winter to early spring (356 mm from June to October 2015). Soil properties (0-10 cm) at the beginning of each sampling period are presented in Table 1.

Table 1. Soil properties (0-10 cm) at the beginning of each sampling period (P1 and P2)

	Clay	**Sand**	**Silt**	**Bulk Density**	**pH**	**OM**	**NH_4^+**	**NO_3^-**
	g/kg			g/cm^3	$CaCl_2$	g/dm^3	mg/kg dry soil	
				P_1*				
$LI_{95\%}$	502	168	330	1.31	5.1	43	283.4	5.0
LI_{Max}	478	172	350	1.30	5.0	46	113.1	8.8
				P_2**				
$LI_{95\%}$	511	179	310	1.32	5.1	43	76.6	20.6
LI_{Max}	487	193	320	1.44	5.1	53	318.4	1.4

* Sampling on 01/08/2016
** Sampling on 02/25/2016

4.2.2. Treatments and experimental design

The two treatments were pre-grazing targets of either 95% or maximum canopy light interception during regrowth ($LI_{95\%}$ and LI_{Max}, respectively). The 2.5 ha experimental area was divided into two farmlets of 18 paddocks (686 m^2 on average) each, according to a randomized complete block design, with six replications. The slope and chemical soil characteristics were considered as blocking criteria.

Treatments based on canopy light interception resulted in contrastant sward structures and determined pre-grazing sward surface heights (SSH) of 100 cm ($LI_{95\%}$) and 135 cm (LI_{Max}). For both pre-grazing SSH, the herbage depletion level (post-grazing height) corresponded to 50% of the pre-grazing SSH as a means to maintain high short-term rates of herbage intake (Fonseca et al., 2012; Carvalho, 2013). Treatments were allocated to the farmlets in mid-January 2015 after grazing and mowing at 45-cm for standardization. During 11-months prior to field measurements, each farmlet was adapted to its respective grazing management strategy. Paddocks were rotationally grazed by 10-13 dairy cows in order to keep grazing management targets. The adaptation period was necessary to adapt sward structure to treatments and to identify the corresponding pre-grazing SSH for the LI pre-grazing targets used ($LI_{95\%}$ and LI_{Max}) (Congio et al., 2018).

Measurements were performed after the adaptation period during the second rainy season from December 2015 to April 2016 (experimental period). A total of 215 kg N/ha (as urea, 45% of N) was applied throughout the experimental period. Because grazing interval was not constant (as a consequence of experimental design), the total amount of N to be applied was divided throughout the experimental period (119 days) and a daily rate of N fertilizer was calculated. The amount of N applied per paddock after each grazing was proportional to the length of the corresponding rest period (daily rate × rest period), ensuring similar N fertilizer application to both treatments at the end of the experimental period (Da Silva et al., 2017).

4.2.3. Soil flux measurements, analysis and flux calculation

Soil gaseous fluxes were measured using the non-ventilated closed static chamber methodology updated by the Global Research Alliance on Agricultural Greenhouse Gases (de Klein and Harvey, 2015). Gas samples were collected during two sampling periods throughout the experimental period (P_1 = 01/08/2016 to 01/22/2016 and P_2 = 02/25/2016 to 03/10/2016). Measurements were made at post-grazing, immediately after N fertilization with ten chambers randomly placed 5-cm into bare ground in each paddock.

Chambers of 17.67 L were made of PVC, composed of a base (30 cm diameter and 20 cm height) plus cap (30 cm diameter and 10 cm height), and were insulated with thermal blanket to avoid heating during sampling (de Klein et al., 2014; Di et al., 2016). Gas samples were collected immediately after chamber closing, and at 30 and 60 min. Samples were collected from cap sampling port using 20 mL plastic syringes (Becton Dickinson, Franklin Lakes, NJ, EUA) and precision glide needles (0.8 × 40 mm; BD), and injected into sealed and evacuated 10 mL glass sample vials. Gas sampling started 24 h after chamber placement to allow soil microbial community to stabilize and minimize overestimation or underestimation of emissions (Chiavegato et al., 2015). Samples were performed during five consecutive days, and then every five days until the 15th day after fertilization. Chambers were removed after P_1 evaluation and re-placed at the beginning of P_2. All samples were collected from 8 to 9:15 am (Alves et al., 2012) and analyzed using gas chromatography at the Laboratory of Analytical Chemistry (Embrapa Pecuária Sudeste, São Carlos, SP, BRA).

The chromatograph GC-2014 (Shimadzu, Columbia, MD, EUA) was equipped with electron capture detectors (ECD) at 325 ºC (column HayeSep T 80/100) for N_2O and flame ionization detectors (FID) at 250 ºC for CO_2 (column HayeSep T 80/100). Calibration curves were established using standard certified gases for CO_2 (260.2 ± 0.68%; 508.3 ± 0.61%, 1058 ± 1.37% and 1995 ± 0.54% ppm) and N_2O (257.3 ± 0.76%; 502.8 ± 0.69%, 999.5 ± 1.77% and 2328 ± 4.84% ppt). Gas chromatography outputs were analyzed to determine linearity from 0 to 60 min. A strong linear relationship was observed for N_2O ($r^2 = 0.88$) and the hourly gas fluxes were calculated according to the increase of gas concentration into the head space over sampling time (de Klein et al., 2014; Luo et al., 2018):

$$Gas\, flux = \frac{\delta Gas}{\delta T} \times \frac{M}{Vm} \times \frac{V}{A} \qquad (1)$$

where δGas is the increase in head space gas concentration overtime (µL/L); δT is the enclosure period (hours); M is the molar weight of N in N_2O; Vm is the molar volume of gas at the sampling temperature (L/mol); V is the headspace volume (m^3); and A is the area covered (m^2). Fluxes were corrected for chamber bias to account for suppression of the surface-atmosphere concentration gradient (Venterea, 2010) and hourly fluxes were assumed to represent mean daily fluxes (de Klein et al., 2014).

4.2.4. Weather and ancillary measurements

Atmospheric pressure, ambient temperature, and rainfall were daily monitored at the weather station located at 50 m from the experimental area. Soil and head-space temperature were recorded for each chamber in each timepoint with a digital thermometer (TE-300, Instrutherm, São Paulo, SP, BRA). At the first day of each sampling period, four cores of each paddock were collected to determine soil bulk and soil particle densities. During the first day of sampling, additional soil samples were taken at 5-cm depth adjacent to each chamber in order to determine soil nitrate (NO_3^-) and ammonium (NH_4^+). Soil N was extracted for one hour with 2 *M* KCl, filtered (Whatman 42) and samples were analyzed for mineral N concentration by flow injection analysis (ASIA; Ismatec, Zürich, Switzerland). At each sampling day prior to gas collection, soil samples were taken (0-5 cm) from adjacent area of each chamber for soil gravimetric moisture determination (24 h at 105 ºC). Volumetric water contents were calculated by multiplying gravimetric water contents by soil bulk density and soil water-filled pore-space (WFPS) was calculated by dividing volumetric water content by total soil porosity (de Klein et al., 2014; Luo et al., 2018).

4.2.5. Statistical analysis

Analysis of variance was performed using the Mixed Procedure of SAS (SAS 9.3; SAS Institute Inc., Cary, NC). Different structures of the variance-covariance matrices were tested, and variance components matrix was chosen as the best fit for the majority of variables based on the Bayesian Information Criterion. The model included fixed effects of treatment, sampling period, and their interaction, and random effect of chamber. Chambers were considered experimental units and sampling periods were treated as repeated measures. Soil temperature, air temperature, WFPS, soil NH_4^+ and soil NO_3^- were tested as explanatory variables. Means were calculated using the LSMEANS statement, compared using the Student's t-test and differences were declared significant at $P \leq 0.05$. For N_2O fluxes, WFPS was used as covariate. To better understand the relations among dependent variables, a principal component analysis (PCA) was performed using a data set comprised of N_2O fluxes, soil NH_4^+, soil NO_3^-, soil temperature, chamber temperature, and WFPS. Principal components scores were submitted to ANOVA to describe and interpret the effects of treatment and periods (Jolliffe, 2002).

4.3. Results

4.3.1. Weather conditions

Weather conditions during the two sampling periods are presented in Figure 1. Air temperature ranged from 16.6 to 35.2 ºC with average of 25.7 ºC during P_1 (Figure 1A). Similarly, during P_2, air temperature ranged from 18.4 to 33.3 ºC with average of 24.9 ºC (Figure 1B). Average soil temperatures were 22.7 and 24.7 ºC for P_1 and P_2, respectively. Accumulated rainfall was 199 mm during P_1 and 106 during P_2 (Figures 1A and 1B, respectively).

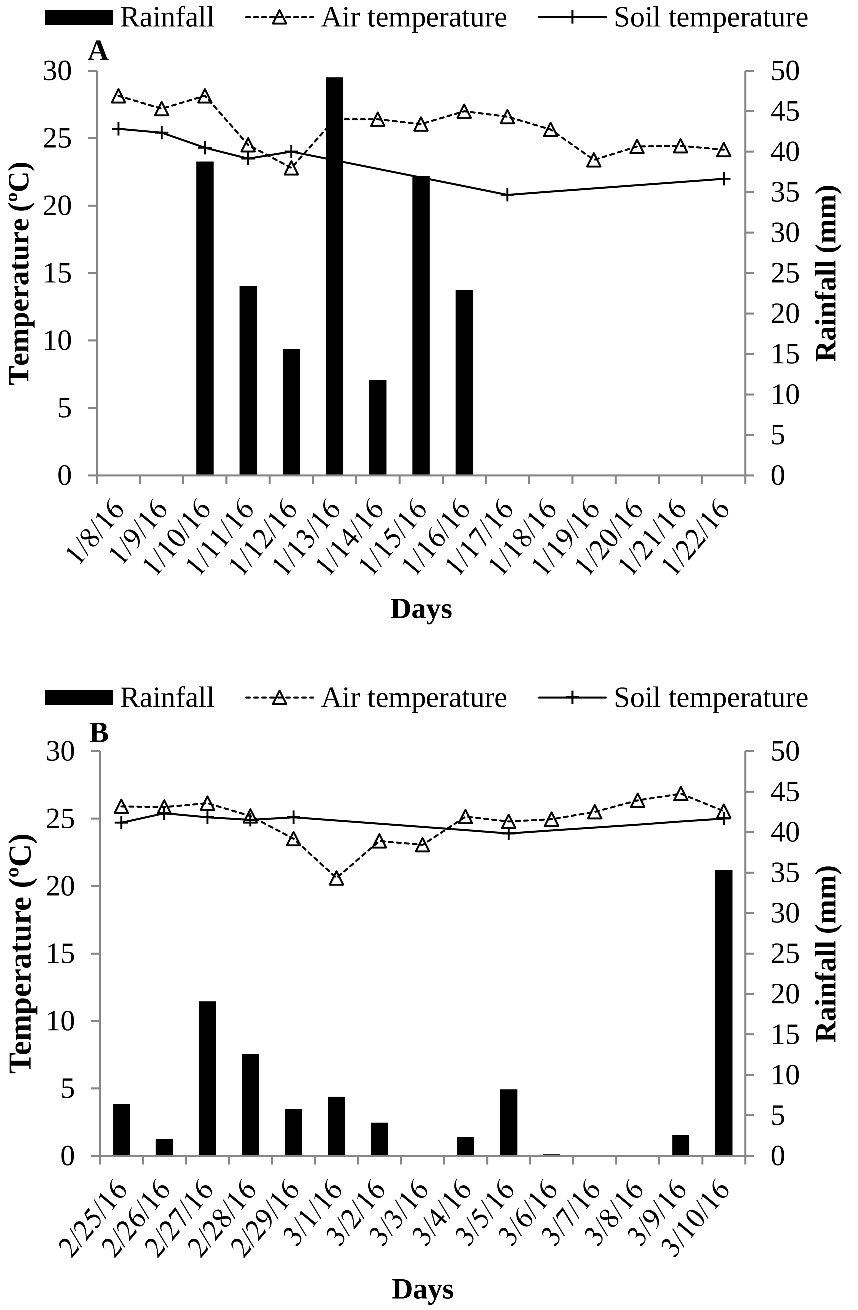

Figure 1. Air and soil (0-5 cm) temperatures (ºC) and rainfall (mm) during sampling periods P_1 (A) and P_2 (B) at the study site (Jan-Mar 2016).

4.3.2. Soil parameters

Water-filled pore space, soil and chamber temperatures were affected by sampling period ($P < 0.01$) being greater for P_2 than P_1 (Table 2). Both soil NH_4^+ and NO_3^- concentrations were not affected by treatment or sampling period ($P > 0.05$), however there was a significant interaction between treatments and sampling period for soil NH_4^+ ($P = 0.0006$) and a trend for soil NO_3^- ($P = 0.0725$). During P_1, there was an effect of LI pre-grazing targets on soil NH_4^+, with greater values observed for $LI_{95\%}$ than LI_{Max}; however, during P_2 soil NH_4^+ was greater for LI_{Max} than $LI_{95\%}$ ($P <$

0.05). Water-filled pore space and rainfall patterns for both periods are presented in Figure 2. Days with no rainfall markedly decreased WFPS during the beginning and the end of P$_1$ (Figure 2A). There was no effect of LI pre-grazing target on WFPS during P$_1$ (*P* = 0.9967; Figure 2A), but the effect was significant during P$_2$ (*P* = 0.05; Figure 2B).

Table 2. Water-filled pore space (WFPS), soil temperature, chamber temperature, soil ammonium and nitrate concentrations from soil established with elephant grass subjected to strategies of rotational stocking management (LI$_{95\%}$ or LI$_{\text{Max}}$) during sampling periods P$_1$ (01/08/2016 to 01/22/2016) and P$_2$ (02/25/2016 to 03/10/2016) (n = 10)

	Period		**SEM^1**	***P*-value**		
	1	**2**		**Trt^2**	**Per^3**	**Trt×Per**
	WFPS, %					
LI$_{95\%}$ LI$_{\text{Max}}$	77.8	94.5	1.57	0.1654	<0.0001	0.1672
	Soil Temp., ºC					
LI$_{95\%}$ LI$_{\text{Max}}$	23.7	24.9	0.11	0.4125	<0.0001	0.4631
	Chamber Temp., ºC					
LI$_{95\%}$ LI$_{\text{Max}}$	22.6	23.7	0.14	0.7344	<0.0001	0.8221
	NH_4^+, mg/kg dry soil					
LI$_{95\%}$	283.4 Aa	76.6 Bb	69.44	0.8771	0.4915	0.0006
LI$_{\text{Max}}$	21.4 Bb	318.4 Aa				
	NO_3^-, mg/kg dry soil					
LI$_{95\%}$	5.0 Aa	20.6 Aa	6.18	0.2218	0.5126	0.0725
LI$_{\text{Max}}$	8.8 Aa	1.4 Ba				

Means followed by the same capital letter in columns and the lower case letter in rows do not differ (*P* > 0.05)

[1] Standard error of the mean

[2] Treatment effect

[3] Sampling period effect

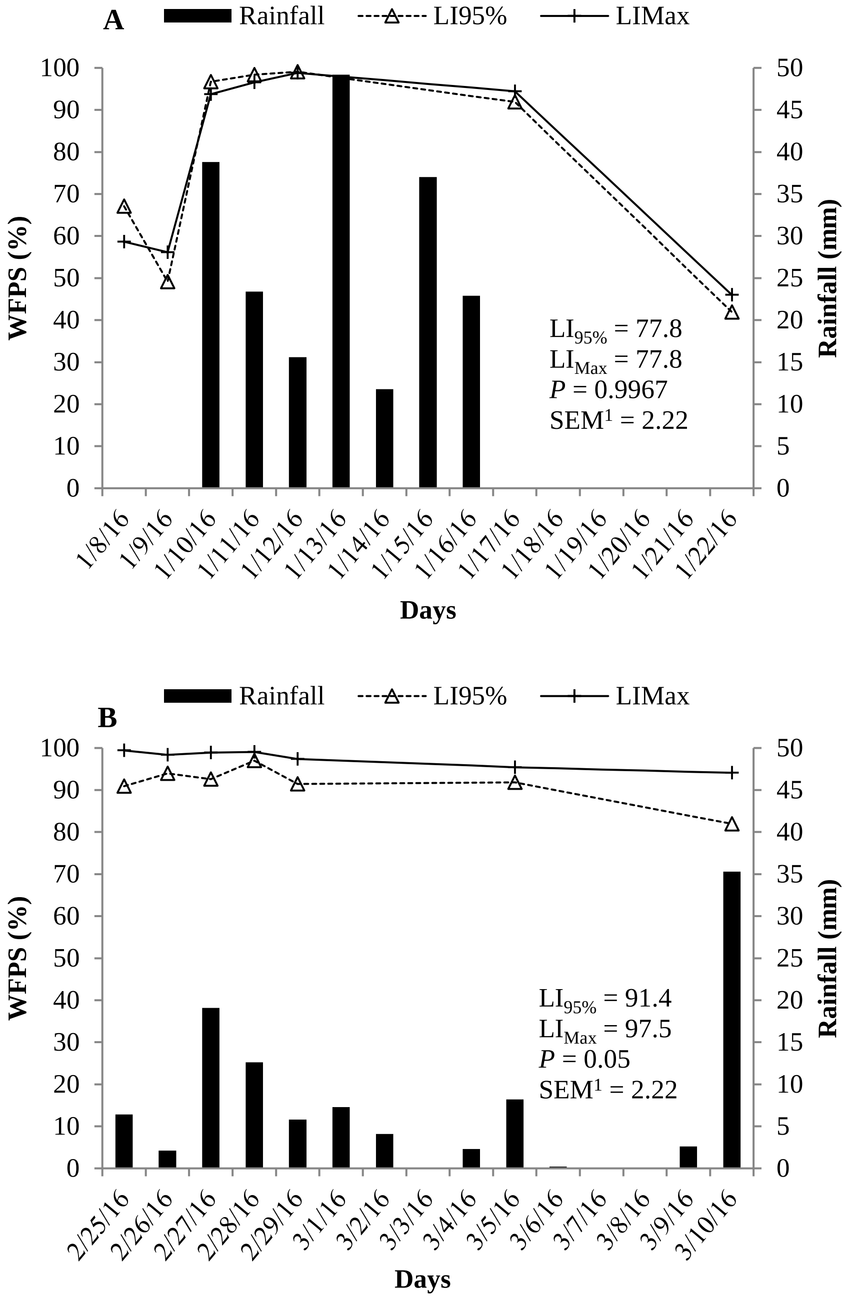

Figure 2. Water-filled pore space (WFPS) and rainfall (mm) during sampling periods P_1 (A) and P_2 (B) at the study site (Jan-Mar 2016).
[1]Standard error of the mean

4.3.3. Nitrous oxide fluxes

Nitrous oxide fluxes were not affected by pre-grazing targets ($P = 0.9975$), however they were strongly affected by sampling period and WFPS ($P < 0.01$; Table 3). On average, N_2O fluxes were greater during P_1 than P_2 (375.9 vs. 134.5 µg $N\text{-}N_2O/m^2.h$; $P < 0.01$). There was a significant interaction between treatments and sampling period ($P = 0.004$). During P_1, N_2O fluxes were greater for $LI_{95\%}$ ($P = 0.0405$) and during P_2 fluxes were greater for LI_{Max} ($P = 0.0414$). Nitrous oxide fluxes across sampling periods and days are shown in Figure 3. Across days, there were no differences on N_2O fluxes during P_1 ($P > 0.05$; Figure 3A). During P_2, four of seven days had greater N_2O fluxes for LI_{Max} than $LI_{95\%}$ ($P < 0.05$; Figure 3B).

Table 3. Nitrous oxide fluxes (µg N-$N_2O/m^2.h$) from soil established with elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}) during sampling periods P_1 (01/08/2016 to 01/22/2016) and P_2 (02/25/2016 to 03/10/2016) (n = 10)

	Period		**SEM**[1]	***P*-value**			
	1	**2**		**Trt**[2]	**Per**[3]	**Trt×Per**	**WFPS**[4]
$LI_{95\%}$	432.8 Aa	77.6 Bb	40.61	0.9975	<0.0001	0.004	<0.0001
LI_{Max}	319.1 Ba	191.5 Ab					

Means followed by the same capital letter in columns and the lower case letter in rows do not differ ($P > 0.05$)

[1] Standard error of the mean

[2] Treatment effect

[3] Sampling period effect

[4] Water-filled pore space effect

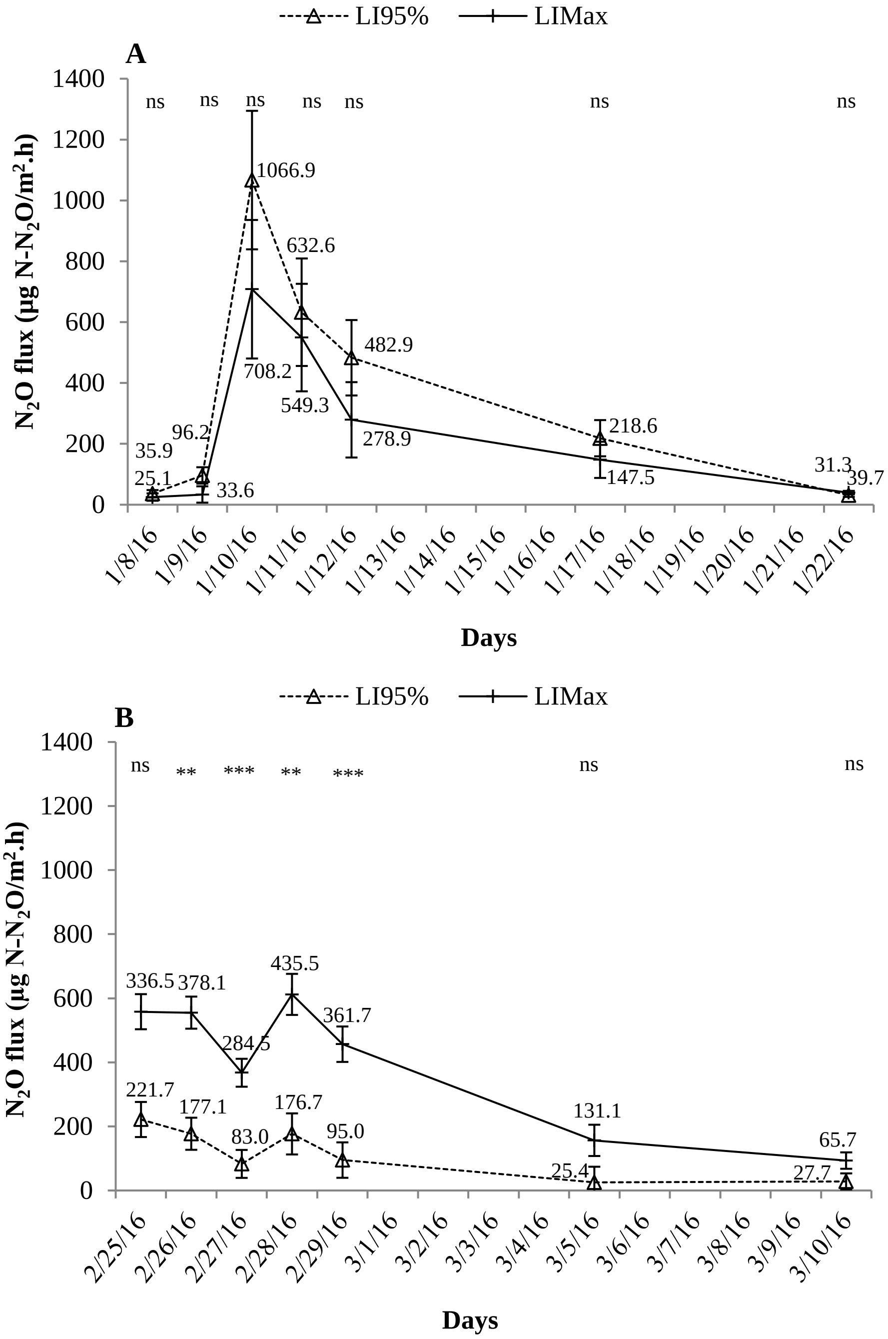

Figure 3. Nitrous oxide fluxes (µg N-N_2O/m^2.h) derived from soil established with elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}) during sampling periods P_1 (A) and P_2 (B).

4.3.4. Principal component analysis

Principal component analysis generated six principal components, however, only the first two were explored because had eigenvalues greater than 1 (Kaiser criteria; Jolliffe, 2002) and accounted for 71.8% of the total variance in N_2O fluxes (Table 4). The first principal component (PC1) explained 49% of the total variance and indicated high positive scores for N_2O fluxes and WFPS, and high negative scores for soil and chamber temperatures. Analysis of variance on PC1 scores showed a significant effect of sampling period ($P < 0.01$). The second principal component (PC2) accounted for 22.8% of the total variance and showed high positive score for soil NH_4^+ and high negative score for soil NO_3^- contents. Analysis of variance on PC2 scores showed a significant effect of treatment × sampling period interaction ($P = 0.0015$).

Table 4. Coefficients of principal components based on the correlation matrix for N_2O fluxes, soil NH_4^+ and NO_3^-, soil and chamber temperatures, and water-filled pore space from soil established with elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max})

Variables	**PC1**	**PC2**
N_2O fluxes	0.49	-0.08
Soil NH_4^+	0.13	0.67
Soil NO_3^-	0.00	-0.70
Soil temperature	-0.48	0.19
Chamber temperature	-0.49	0.07
Water-filled pore space	0.52	0.15
Eigenvalue	2.94	1.37
% of variation explained	49.0	22.8
ANOVA	***P*-value**	
Trt[1]	0.1149	0.6239
Per[2]	<0.0001	0.2950
Trt×Per	0.6934	0.0015

[1] Treatment effect
[2] Sampling period effect

4.4. Discussion

The grazing management strategies used in this study provided contrastant pre- and post-grazing SSH that affected grazing interval and ultimately the number of grazing cycles. For LI_{Max}, pre- and post-grazing SSH were 135 and 64 cm, respectively, which resulted in an average grazing interval of 32 days and 3.5 grazing cycles during the experimental period (Congio et al., 2018). On the other hand, for $LI_{95\%}$, pre- and post-grazing SSH were 100 and 50 cm, respectively, which resulted in an average grazing interval of 21 days and 5.6 grazing cycles (Congio et al., 2018). Considering adaptation and experimental periods (from January 2015 to April 2016) there were 9.3 grazing cycles for LI_{Max} and 14.1 for $LI_{95\%}$, indicating greater frequency of defoliation on paddocks managed with the $LI_{95\%}$ target relative to those managed with the LI_{Max} target. To keep the pre- and post-grazing targets, stocking rate was 33% greater for $LI_{95\%}$ than LI_{Max} during the experimental period (9.3 vs. 7.0 cows/ha; Congio et al., 2018). These grazing conditions created different scenarios of intensification, solely by changing pre-grazing targets ($LI_{95\%}$ or LI_{Max}). It is worthwhile to mention that the greater stocking rate obtained in $LI_{95\%}$ was supported by greater leaf accumulation and greater grazing efficiency rather than increased N fertilizer input, usually applied in intensive temperate pasture-based systems (Ramsbottom et al., 2015; Macdonald et al., 2017; Congio et al., 2018).

Emissions of N_2O are a result of soil microbial nitrification and denitrification (de Klein and Eckard, 2008; Saggar et al., 2013). Both processes are mediated by soil properties such as mineral N (i.e. NH_4^+ and NO_3^-) and organic carbon availabilities, moisture, pH, temperature, and texture (de Klein et al., 2008; Luo et al., 2017). In grazed pastoral soils, the factors pointed out as key drivers of N_2O fluxes are N inputs (i.e. urine patches and fertilizer) and WFPS (de Klein et al., 2008; Luo et al., 2017). Nitrous oxide fluxes and soil NH_4^+ varied with LI pre-grazing target × sampling period interaction, while a trend was observed for the effect of NO_3^-. On the other hand, the variables related to weather (i.e. WFPS, soil and chamber temperatures) varied only with sampling period. Most studies have indicated that high N_2O emissions are usually associated with anaerobic soils with enough NO_3^- supply suggesting that denitrification is the main process responsible for N_2O emissions (de Klein and Eckard, 2008; de Klein et al., 2008). However, on excessively saturated soils with higher WFPS (i.e. optimal conditions for denitrification), as observed in P_2, denitrification is complete and results in greater N_2:N_2O ratio (Bolan et al., 2004; de Klein et al., 2008). Although the accumulated rainfall was greater during P_1 (199 mm) than P_2 (106 mm), the WFPS was constantly greater throughout P_2 than P_1. These results are likely associated with better rainfall distribution during P_2, where there were 80% of rainy days, while during P_1 there were just 47% of rainy days.

The WFPS oscillation throughout P_1 followed the rainfall pattern. At day 3 (1/10/16), a 39 mm rainfall increased WFPS from around 50 to more than 90% (Figure 2A), driving N_2O fluxes up to 1000 μg N-N_2O/m^2.h (Figure 3A). Thenceforward, the WFPS was kept above 90% until day 10 (1/17/16) and N_2O fluxes decreased likely because of low oxygen availability that may have favored complete denitrification and N_2 production (Bolan et al., 2004; de Klein et al., 2008). Throughout P_2, the more uniform rainfall regime maintained WFPS above 90% with little oscillation until day 15 (3/10/16; Figure 2B), and N_2O fluxes were kept moderate during the first half of P_2, decreasing at the end of the period (Figure 3B). Studies have reported that the peak of N_2O emissions occurs at WFPS values around 60-80%, when simultaneous nitrification and denitrification were at maximum levels (Davidson, 1992; Rafique et al., 2011). Above this WFPS range, denitrification is the main source of N_2O and under excessively anaerobic conditions, N_2:N_2O ratio remains greater (Bolan et al., 2004; de Klein et al., 2008; Rafique et al., 2011). The results of PCA pointed to an interaction among the driving factors regulating N_2O fluxes from soil. The first principal component indicated that environmental factors (i.e. WFPS, soil and chamber temperatures) were determinants of N_2O emissions and explained 49% of the whole dataset variability. Principal component two showed that factors related to LI pre-grazing targets (i.e. soil NH_4^+ and NO_3^-) had the highest scores and accounted for 22.8% of total variance. Flechard et al. (2007) also reported that weather factors explained half of the total variability in their N_2O flux dataset of ten sites for three years across Europe. Analysis of variance on PC1 and PC2 scores corroborated the results from the analysis of variance, where environmental factors showed significant effect for sampling period, as observed in PC1, and treatment related factors showed significant effect for the LI pre-grazing target × sampling period interaction, as observed in PC2.

Both soil NH_4^+ and NO_3^- represented the concentration immediately after urea fertilization at day one, and therefore indicate N availability at the beginning of each sampling period. For both LI pre-grazing targets, a total of 215 kg N/ha was applied throughout the experimental period. However, this amount was divided in 3.5 and 5.6 instalments for LI_{Max} and $LI_{95\%}$, respectively. Therefore, the N inputs from urea fertilizer immediately before N_2O sampling were greater for LI_{Max} than $LI_{95\%}$ during P_1 (75 vs. 44 kg N/ha) and P_2 (111 vs. 57 kg N/ha). However, there was a significant treatment × sampling period interaction on soil NH_4^+ concentration, most likely associated with urinary-N discharge. During P_1, there was a greater urinary-N discharge for $LI_{95\%}$ than LI_{Max} (26.3 vs. 20.9 kg of urinary-N/paddock) caused by higher stocking rate (10.0 vs. 8.3 cows/ha), which resulted in greater N_2O fluxes for $LI_{95\%}$. Inversely, during P_2, the soil NH_4^+ and N_2O fluxes were greater for LI_{Max} than $LI_{95\%}$. During this period, the greater urinary-N discharge (46.8 vs. 44.8 kg of urinary-N/paddock) was likely associated with greater stocking period (1.88 vs. 1.46 days) for LI_{Max} relative to $LI_{95\%,}$ since both treatments had similar stocking rate (9.5 vs. 9.9 cows/ha). These results are in agreement with most studies that have reported urine patches as the main source of N_2O from grazed pasture soil mainly by providing highly localized concentrations of available N, ranging from 200-2000 kg N/ha, associated with increased moisture and temperature conditions (Selbie et al., 2015; Luo et al., 2018).

Dairy farming systems based in temperate pastures are usually more intensive than tropical pasture-based dairy systems (Congio et al., 2018). Temperate forage crops were deeply studied and the understanding of their ecophysiology allowed for better use by farmers through adoption of adequate grazing management strategies, ensuring high milk productivity. The intensification of such systems is usually coupled with extra inputs of N fertilizer to boost forage growth or external supplementary feed, both aiming at increased stocking rate (Ramsbottom et al., 2015; Macdonald et al., 2017). In the tropics, dairy farming systems usually have low N inputs and adopt inadequate grazing management strategies resulting in low milk productivity. Therefore, the intensification of tropical pasture-based dairy systems is possible through adoption of adequate grazing strategies rather than extra N inputs or additional supplements, provided that minimum soil fertility to meet plant nutritional demand is ensured. The results indicated the opportunity to increase milk productivity in 52% (170 and 112 kg/ha.day for $LI_{95\%}$ and LI_{Max}, respectively; Congio et al., 2018) only with adoption of strategic grazing management (i.e. $LI_{95\%}$ pre-grazing target).

Experimental approaches have shown that intensively managed pastures are greater sources of N_2O than extensively managed pastures (Flechard et al., 2007; Rafique et al., 2011). Rafique et al. (2011) reported that frequently grazed sites that applied 400 kg of N/ha emitted two times more N_2O compared to less frequently grazed sites that used around 300 kg of N/ha. However, in their study, intensively managed systems were generated through greater inputs of N fertilizer. In the present study, the more intensive grazing strategy was obtained through optimization of ecological processes rather than additional inputs of N fertilizer (Congio et al., 2018). Although urinary-N excretion has increased soil NH_4^+ and ultimately N_2O fluxes during P_1 for $LI_{95\%}$, during P_2 the urinary-N excretion and N_2O fluxes were greater for LI_{Max} counterbalancing the emissions for the entire experimental period (255 µg N-N_2O/m^2.h; $P = 0.9975$). Converting hourly N_2O fluxes to daily basis and relating to milk productivity, $LI_{95\%}$ was 35% more efficient than LI_{Max} considering emissions for the entire period (0.36 vs. 0.55 g N-N_2O/kg milk.ha.day). Even during P_1, when N_2O fluxes were greater for $LI_{95\%}$ than LI_{Max}, $LI_{95\%}$ emited less N-N_2O/kg of milk.ha.day than LI_{Max} (0.61 vs. 0.68 g N-N_2O/kg milk.ha.day). In addition, strategic grazing management decreased 34% urea-N applied per milk yield per hectare (0.57 vs. 0.86 g urea-N/kg milk.ha.day).

In a context where there is concern about the intensification of temperate pasture-based dairy systems through greater N fertilizer inputs, these findings highlight an opportunity to improve the efficiency of tropical pasture-based dairy systems through optimization of ecological processes. Strategic grazing allows for intensification that is not coupled with increases in inputs of external resources (i.e. fertilizer, external supplements) but rather with efficient use of existing resources (i.e. solar radiation, rainwater, pasture, fertilizer, supplement). Congio et al. (2018) have shown that strategic grazing management might reduce approximately 20% of enteric CH_4 emission intensity and CH_4 yield of dairy cows in tropical pasture-based systems. Carbon footprint in dairy farming systems is often dependent on emissions of enteric CH_4 and N_2O but also on carbon sequestration by forage crops with increase in soil organic carbon. Abdalla et al. (2018) revealed that the impact of grazing intensity on soil organic carbon is strongly climate dependent, and moist-warm regions present different responses than dry-warm, dry-cold and moist-cold climates. The authors highlighted that C_4 grass species under high grazing intensities in moist-warm regions are more likely to increase soil organic carbon through enhanced plant turnover (i.e. root and litter) and excreta distribution than C_4 under low grazing intensity. Then, the intensification of tropical pasture-based dairy farms through strategic grazing management uncoupled to N fertilizer increases could be a strategy for GHG mitigation. In addition, strategic grazing management is a noncost and readily available practice with easy adoption that enhances profitability of tropical pasture-based systems.

4.5. Conclusions

Nitrous oxide fluxes from grazed pastoral soils in moist-warm conditions is a very complex process regulated by environmental conditions and soil nitrogen availability. The central hypothesis that frequent defoliation provided by the $LI_{95\%}$ pre-grazing target would result in greater N_2O fluxes from soil than less frequent defoliation (i.e. LI_{Max}) was not confirmed. However, these results associated with those of Congio et al. (2018) highlight that it is possible to intensify tropical pasture-based dairy systems through the adoption of adequate grazing strategies rather than extra N fertilizer inputs or additional supplements, as is usually pointed out for temperate grazing systems. This indicates the opportunity to significantly enhance milk productivity from tropical pasture-based systems using strategic grazing management ($LI_{95\%}$; Congio et al., 2018) and decrease 35% the N-N_2O emitted per kg of milk. However, further farm-scale studies are necessary for a wide range of tropical grazed soils and dairy farming systems, associating not only GHG sources, but also the carbon sequestration by pasture soils, and milk productivity to achieve more accurate estimates of carbon balance and then to support mitigation plans by policy makers.

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5. EFFECTS OF TIMING OF PADDOCK ALLOCATION ON MILK YIELD AND ENTERIC METHANE EMISSIONS FROM DAIRY COWS

Abstract

Dairy products are major components of the human diet. Pasture-based systems are important milk suppliers to dairy industry in temperate and tropical climates and thereby will play relevant role to support the growing demand. However, this additional milk supply must be obtained through higher yields resulting from intensification of existing farming systems using environmentally friendly and economically profitable strategies towards sustainable intensification. The objective of this study was to investigate the influence of timing of paddock allocation (AM or PM) on the nutritive value of rotationally managed elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon), and the dry matter intake (DMI), milk yield, milk composition, and enteric methane (CH_4) emissions of Holstein × Jersey dairy cows. The hypothesis was that new paddock allocation to dairy cows in the afternoon, when herbage has greater nutritive value, increases nutrient intake and milk yield, and reduces enteric CH_4 emissions per kg of milk, relative to paddock allocation in the morning. Herbage sampled in the afternoon had greater dry matter, soluble carbohydrates, starch, and non-fibrous carbohydrate/protein ratio, and lesser neutral-detergent fiber and acid-detergent fiber concentrations. There was no treatment effect on milk yield. However, protein and casein yields tended to be greater for PM than AM. Milk urea nitrogen was greater for cows grazing paddocks allocated during the morning relative to those allocated in the afternoon. The timing of paddock allocation did not affect DMI, daily enteric CH_4 emission, and enteric CH_4 per kg of milk. The results ratify the general understanding of diurnal variation in herbage chemical composition. However, the increase in nutritive value of the afternoon relative to the morning herbage was not enough to increase DMI and milk yield, or to decrease CH_4 emission intensity by the dairy cows as hypothesized. The findings also indicate that new paddock allocation during the afternoon can be a simple and useful grazing strategy that results in greater N partitioning to protein yield, and lower excretion of urea N in milk.

Keywords: Timing of paddock allocation; Enteric methane emissions; Herbage quality; Diurnal variation; Non-fibrous carbohydrate; Elephant grass

5.1. Introduction

Dairy products are major components of the human diet (Aguirre-Villegas et al., 2017). Pasture-based systems are important milk suppliers to dairy industry in temperate (Chapman, 2016; Macdonald et al., 2017) and tropical climates (Santos et al., 2014; de Souza et al., 2017) and thereby will play relevant role to support the growing demand (Godfray et al., 2010; Conforti, 2011; Alexandratos and Bruinsma, 2012). However, this additional milk supply must be obtained through higher yields resulting from intensification of existing farming systems using environmentally friendly (Tilman et al., 2002) and economically profitable (Foote et al., 2015; Gregorini et al., 2017) strategies towards sustainable intensification (Godfray et al., 2010; Congio et al., 2018).

Several studies have reported diurnal variations in herbage chemical composition (Lechtenberg et al., 1971; Orr et al., 1997; Ciavarella et al., 2000; Griggs et al., 2005; Gregorini et al., 2006; Shewmaker et al., 2006; Gregorini et al., 2008; Morin et al., 2011). Such variations were attributed to the balance among processes of plant photosynthesis, plant respiration, and plant transpiration that results in greater non-fibrous carbohydrate (NFC) and dry matter (DM) accumulation from dawn to dusk (Curtis, 1944; Lechtenberg et al., 1971). The increase in NFC and DM concentrations mainly occur in the upper layers of the canopy (Delagarde et al., 2000), often diluting fiber and nitrogen (N) concentrations (Gregorini, 2012; Vibart et al., 2017), and enhancing herbage biomechanical properties (Gregorini et al., 2009) and digestibility (Burns et al., 2007; Pelletier et al., 2010; De Oliveira et al., 2018). Therefore, temporal patterns of herbage intake, ingestive and digestive behavior of grazing ruminants can be altered by timing of new strip or paddock allocation to grazing animals in rotationally managed pastures (Gibb et al., 1998; Orr et al., 2001; Gregorini et al., 2006; Gregorini et al., 2008; Abrahamse et al., 2009; Gregorini, 2012; Pulido et al., 2015; Vibart et al., 2017). Although these studies have not reported that such modifications in the herbage chemical composition can increase daily dry matter intake (DMI), Gregorini (2012) suggested that ruminants moved to a new fresh paddock in the afternoon might increase their nutrient intake because of longer and more intensive grazing during dusk, when herbage nutritive value is at its peak.

Enteric CH_4 is the predominant source of greenhouse gases (GHG) emissions in dairy systems (Crosson et al., 2011; Aguirre-Villegas et al., 2017) and represent more than 80% of total GHG emissions in pasture-based farming systems (Guerci et al., 2013). According to Janssen (2010), the nature and amount of feed (e.g. herbage chemical composition and DMI, respectively) are key determinants of enteric CH_4 emissions from ruminants. Modeling studies have shown possible reductions on enteric CH_4 emissions intensity (g/kg of milk) by dairy cows when herbage NFC increases at the expense of fiber concentrations (Ellis et al., 2012), and Gregorini (2012) suggested the need of field research to assess this hypothesis.

The objective of this study was to investigate the influence of timing of paddock allocation (AM or PM) on the nutritive value of rotationally managed elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon), and the DMI, milk yield, milk composition, and enteric CH_4 emissions of Holstein × Jersey dairy cows. The hypothesis was that new paddock allocation to dairy cows in the afternoon, when herbage has greater nutritive value, increases nutrient intake and milk yield, and reduces enteric CH_4 emissions per kg of milk, relative to paddock allocation in the morning.

5.2. Material and Methods

All procedures for this study were approved by the Animal (15.5.1246.11.2) and Environment Ethics Committees (17.5.999.11.9) at the University of São Paulo, College of Agriculture “Luiz de Queiroz” (USP/ESALQ).

5.2.1. Study site

The experiment was conducted from January to March 2017 in Piracicaba, SP, Brazil (22º42’S, 47º38’W and 546 a.s.l.) on a rainfed, non-irrigated elephant grass pasture (*Pennisetum purpureum* Schum. cv. Cameroon) established in 1972 in a high fertility Eutroferric Red Nitossol. The climate is sub-tropical with dry winters and 1328 mm average annual rainfall (CEPAGRI, 2012). The mean temperature and accumulated rainfall during the experiment were 24.5 ºC and 407 mm respectively.

5.2.2. Treatments and experimental design

The 3.3 ha experimental area was divided up into two farmlets of 24 paddocks each (688 m^2 on average), and managed using a common rotational grazing strategy with one day of occupation. Pre- and post-grazing sward surface heights (SSH) were 100 and 55 cm, respectively, which were found to optimize grazing efficiency and feeding value of elephant grass cv. Cameroon (Congio et al., 2018). Paddocks were subjected to a period of 11 months prior to the beginning of the experiment aiming to adapt sward structure to the grazing strategy used.

The two treatments corresponded to timings of herd allocation to a new paddock, either after morning milking at 6:00 am (AM) or after afternoon milking at 4:00 pm (PM). The experimental design was a randomized complete block, with eight replications, with slope and chemical soil characteristics used as blocking criteria. Each paddock received 56 kg N/ha (as urea) during the experiment splitted in 2 instalments. Fertilizer application was made soon after grazing. The experimental period was divided in two sampling periods of 4 weeks each (P_1 and P_2) and measurements were made during the last 7 days of each sampling period.

5.2.3. Plant measurements

The SSH was measured from ground level to top leaf horizon by 40 systematic readings, using a stick graduated in centimeters (Pereira et al., 2015a; Congio et al, 2018). Pre-grazing herbage mass was quantified in each grazing cycle on three rectangular samples collected randomly (0.94 m^2 each). Herbage was clipped above the target post-grazing SSH, weighed fresh, and sub-sampled to determine plant-part components by hand separation into leaf (leaf blades), stem (stems + leaf sheaths) and dead material (Pereira et al., 2015b; Congio et al, 2018). Herbage allowance was calculated by the relationship between pre-grazing herbage mass (above post-grazing SSH) and number of cows per day (Pérez-Prieto and Delagarde, 2013; Congio et al, 2018).

Herbage samples to determine chemical composition were taken daily during the last 7 days of each sampling period (P_1 and P_2) immediately before herd allocation to paddocks (6 am and 4 pm).

Herbage was clipped above target post-grazing SSH at ten randomized sampling sites per paddock, homogenized, sub-sampled, freeze-dried, and ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA). Dry matter (DM) and ash concentrations were determined at 105 ºC for 24 h and 600 ºC for 4 h, respectively (AOAC International, 2005). Neutral-detergent fiber (NDF), acid-detergent fiber (ADF) and lignin concentrations were determined sequentially (Van Soest et al., 1991). Ether extract (EE) concentration was determined according to AOAC International (2005). Total N concentration was determined by the Dumas combustion method using N analyzer (Leco FP-2000 N Analyzer; Leco Instruments Inc., St. Joseph, MI, USA), and crude protein (CP) concentration calculated as N × 6.25. Neutral-detergent insoluble crude protein (NDICP), acid detergent-insoluble crude protein (ADICP), and soluble N concentrations were analyzed according to Licitra et al. (1996), and N fractions were determined by methodology adapted from Sniffen et al. (1992). Soluble carbohydrates in 80% ethanol-solution (SC) and starch concentrations were determined according to Hall (2003).

5.2.4. Herd and feeding

Twenty Holstein × Jersey dairy cows averaging 461 ± 72 kg body weight (BW) and 2.83 ± 0.23 body corporal score (BCS) were used. Four weeks prior to the experiment, all cows were managed in a single herd grazing elephant grass cv. Cameroon and receiving 6 kg (fresh basis) of commercial concentrate daily. Cows were then stratified, grouped in pairs and allocated to 10 blocks according to pre-experimental milk yield (18.6 ± 4.6 kg/d) and days in milk (102 ± 82 DIM). Within pairs, cows were randomly assigned to treatments (AM and PM).

Concentrate meals were fed individually twice daily (4:30 am and 2:30 pm) before milking (5 am and 3 pm) at a rate of 1 kg of concentrate/3 kg of milk (considering the average of each block). The rate was established based on milk yield at the beginning of each period (Danes et al., 2013). The concentrate meal was composed of fine ground corn (80%), soybean meal (15%) and mineral (5%), with chemical composition as following: 86.8% of DM, 9.4% of ash, 13.6% of CP, 13.2% of NDF, 3.4% of ADF, 3.9% of EE and 59.9% of NFC.

5.2.5. Animal measurements

Cows were weighed and BCS recorded at the end of each sampling period (P_1 and P_2) during three consecutive days (Edmonson et al., 1989). Milk yield was recorded daily with samples collected in vials containing bronopol preservative pill and analyzed for fat, protein, lactose, milk solids and milk urea nitrogen (MUN) using infrared procedures (MilkoScan FT+; Foss North America Inc., Eden Prairie, MN).

Herbage intake was estimated from total fecal excretion and feed indigestibility. To estimate total fecal excretion, titanium dioxide (TiO_2) was dosed twice daily (20 g/cow per day) after concentrate meals during 12 days. Fecal samples were collected from rectum following concentrate meals during the last 5 days, dried in a forced-air drier at 55 °C for 72 h, ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA), and composited into one sample per measurement period by cow. Titanium dioxide concentration in feces was determined according to Myers et al. (2004). To determine the feed indigestibility, the indigestible NDF (iNDF) content of herbage, concentrate, and fecal samples were estimated by 240 h in vitro incubation (Goeser and Combs, 2009). Total fecal excretion, fecal excretion from concentrate, and herbage intake were calculated according to de Souza et al. (2015).

Enteric CH_4 emissions were estimated using sulfur hexafluoride (SF_6) as tracer gas (Johnson and Johnson, 1995). Pre-calibrated permeation tubes containing SF_6 with known release rates (1.48 ± 0.32 mg/d) were placed into the rumen of each cow. Sampling apparatus included a PVC collection canister (2.3 L), and adjustable halter containing stainless steel capillary tubing and brass connections. Canisters were vacuumed to approximately -13.5 psi using a three-stage vacuum pump (Symbol, Sumaré, SP, Brazil) and Druck DPI 705 digital manometer (GE Druck, South Burlington, VT, EUA) and replaced daily just after the afternoon concentrate meal. Cows were adapted to the sampling apparatus during 7 days prior to collection. Enteric CH_4 emissions were measured at 24-hour intervals over 7 consecutive days. Background SF_6 and CH_4 concentrations were determined using two sampling apparatus placed daily in the field near the grazing herd. Prior to chromatograph determination, canisters were pressurized to 1.3-1.5 psi with ultrapure nitrogen 5.0, and pressures recorded by Druck DPI 705 digital manometer (GE Druck, South Burlington, VT, EUA) in order to calculate the dilution factor. Methane and SF_6 concentrations were determined at the Laboratory of Biogeochemistry and Tracer Gases Analysis (Embrapa Meio Ambiente, Jaguariúna, SP, BRA) using gas chromatography (HP6890, Agilent, Delaware, USA). Chromatograph was equipped with flame ionization detector (FID) at 280°C for CH_4 (column megabore, 0.53 mm × 30 m × 15µm, Plot HP-Al/M), and electron capture detector (ECD) at 300°C for SF_6 (column megabore, 0.53 mm × 30 m × 25 µm, HP-MolSiv), with two loops of 0.5 cm^3 maintained at 80 °C attached to two six-way valves. Calibration curves were established using standard certified gases for CH_4 (4.85 ± 5%; 9.96 ± 1.65% and 19.1 ± 3.44% ppm) and SF_6 (34.0 ± 9.0; 91.0 ± 9.0 and 978.0 ± 98.0 ppt) (Westberg et al., 1998). Daily methane emissions were calculated from collected SF_6 and CH_4 concentrations in the canisters discounting background concentrations, and value of SF_6 permeation tube release rate (Johnson and Johnson, 1995).

5.2.6. Statistical analysis

Analysis of variance was performed using the Mixed Procedure (SAS 9.3; SAS Institute Inc., Cary, NC). Different structures of the variance-covariance matrices were tested and Bayesian Information Criterion was adopted to select the best fit matrix. For plant parameters analysis, paddock was considered as experimental unit, and for animal measurements, cow was considered as experimental unit. Cows or paddocks blocks were considered random terms, and timing of new paddock allocation, sampling period and their interactions were treated as fixed effects. Sampling periods were treated as repeated measures. Means were calculated using the LSMEANS statement and compared using the Student's *t*-test. Differences were declared significant at $P \leq 0.05$, and trends were declared at $P \leq 0.10$.

5.3. Results

5.3.1. Sward characteristics

Sward characteristics are presented in Table 1. Both pre- and post-grazing SSH did not vary between treatments ($P = 0.3124$ and $P = 0.8619$, respectively). Post-grazing SSH was greater during P_1 than during P_2 ($P = 0.0127$; 56.8 vs. 53.2 cm, respectively). There was no effect of timing of paddock allocation on pre-grazing herbage mass ($P = 0.6742$), leaf-to-stem ratio ($P = 0.9214$) and herbage allowance ($P = 0.7694$).

Table 1. Pre- and post-sward surface height (SSH) (cm), pre-grazing herbage mass (kg of DM/ha), leaf:stem ratio and herbage allowance (kg of DM/cow.day) of rotationally managed elephant grass cv. Cameroon with new paddocks allocated to dairy cows either in the morning (AM) or in the afternoon (PM) (n = 8)

Item	**Treatments**		**SEM**[1]	***P*-value**		
	AM	**PM**		**Trt**[2]	**Per**[3]	**Trt×Per**
Pre-SSH	101.6	100.4	0.80	0.2770	0.5879	0.7848
Post-SSH	55.7	54.2	1.18	0.2945	0.0127	0.9889
Pre-grazing herbage mass[4]	2270	2180	208.5	0.6742	0.6812	0.8185
Leaf:Stem ratio[4]	73.6	75.5	30.50	0.9214	0.9566	0.1092
Herbage allowance[4]	15.4	14.9	1.33	0.7694	0.8090	0.4176

[1] Standard error of the mean
[2] Treatment effect
[3] Sampling period effect
[4] Estimated above post-grazing SSH

5.3.2. Herbage chemical composition

Overall, herbage chemical composition differed between treatments (Table 2). Herbage sampled in the afternoon had greater DM ($P = 0.0003$), SC ($P < 0.01$), starch ($P < 0.01$), NDICP ($P =$

0.0102), NFC/PROT ratio ($P < 0.01$), and lesser NDF ($P = 0.0127$) and ADF ($P = 0.0053$) concentrations. There was no treatment effect on OM ($P = 0.7879$), lignin ($P = 0.8951$), EE ($P = 0.6610$), CP ($P = 0.3324$), soluble and degradable protein ($P = 0.2106$ and $P = 0.7746$, respectively), and ADICP ($P = 0.7893$) concentrations. During P_2, DM ($P = 0.0007$), OM ($P < 0.0001$), NDF ($P < 0.0001$) and NDICP concentrations ($P < 0.0001$) were greater, and EE ($P = 0.0216$) and degradable protein ($P = 0.0064$) concentrations were lower relative to P_1. There were no interactions between treatments and sampling periods.

Table 2. Herbage chemical composition (% of DM) of rotationally managed elephant grass cv. Cameroon with new paddocks allocated to dairy cows either in the morning (AM) or in the afternoon (PM) (n = 7)

Item	**Treatments**		**SEM**[1]	***P*-value**	**Periods**		***P*-value**
	AM	**PM**			**1**	**2**	
Dry matter	18.9	22.2	0.67	0.0003	19.1	22.1	0.0007
Organic matter	90.5	90.6	0.42	0.7879	89.1	92.0	<0.0001
Soluble carbohydrates	5.4	8.2	0.27	<0.0001	6.8	6.8	0.8955
Starch	1.5	2.9	0.13	<0.0001	2.3	2.1	0.1571
Neutral detergent fiber	61.8	60.0	0.56	0.0127	59.2	62.5	<0.0001
Acid detergent fiber	36.8	34.5	0.54	0.0053	35.7	35.6	0.9287
Lignin	3.4	3.4	0.16	0.8951	3.5	3.2	0.1237
Ether extract	3.1	3.0	0.08	0.6610	3.1	2.9	0.0216
Crude protein	17.6	17.1	0.42	0.3324	17.7	17.0	0.2506
Protein fractions[2]**, % of CP**							
Soluble protein	26.0	24.0	1.11	0.2106	23.9	26.1	0.1869
Degradable protein	51.9	51.5	1.00	0.7746	53.8	49.5	0.0064
NDICP	16.0	17.5	0.37	0.0102	15.5	18.0	<0.0001
ADICP	6.6	6.6	0.31	0.7893	6.7	6.4	0.4115
NFC/PROT[3]	0.52	0.87	0.040	<0.0001	0.68	0.70	0.6847

[1] Standard error of the mean corresponds to both treatment and period effects

[2] Protein fractions adapted from Sniffen et al. (1992): Soluble protein (A+B1), Degradable protein (B2), NDICP (B3) and ADICP (C)

[3] NFC: (soluble carbohydrates + starch); PROT: (soluble protein + degradable protein)

5.3.3. Animal performance

The effects of timing of paddock allocation on animal performance are shown in Table 3. There was no treatment effect on milk yield ($P = 0.6618$), fat yield ($P = 0.9181$), and milk solids yield ($P = 0.9240$). However, protein ($P = 0.0899$) and casein ($P = 0.0632$) yields tended to be greater for PM than AM. Timing of paddock allocation did not affect milk fat ($P = 0.6285$), milk protein ($P = 0.2976$), milk casein ($P = 0.2346$), and milk solids ($P = 0.6760$) concentration. Milk lactose concentration ($P = 0.0003$) and MUN ($P = 0.0032$) were greater for cows grazing paddocks allocated during the morning relative to those allocated in the afternoon.

Table 3. Milk yield (kg/d) and milk composition (% unless specified otherwise) of dairy cows grazing rotationally managed elephant grass cv. Cameroon with new paddocks allocated either in the morning (AM) or in the afternoon (PM) (n = 10)

Item	**Treatments**		**SEM**[1]	***P*-value**		
	AM	**PM**		**Trt**[2]	**Per**[3]	**Trt×Per**
Yield						
Milk	17.2	17.4	1.20	0.6618	0.1023	0.8792
Fat	0.59	0.60	0.03	0.9181	0.2626	0.5564
Protein	0.55	0.58	0.02	0.0899	0.1802	0.3715
Casein	0.42	0.45	0.02	0.0632	0.1021	0.0979
Milk solids	2.1	2.1	0.11	0.9240	0.0422	0.8164
Composition						
Fat	3.5	3.5	0.13	0.6285	0.9415	0.2179
Protein	3.2	3.3	0.10	0.2976	0.0373	0.1190
Lactose	4.6	4.4	0.06	0.0003	0.1067	0.1627
Casein	2.5	2.6	0.09	0.2346	0.1945	0.0295
Milk solids	12.3	12.2	0.25	0.6760	0.3864	0.8089
MUN[4], mg/dL	14.6	13.0	0.46	0.0032	0.0538	0.217

[1] Standard error of the mean
[2] Treatment effect
[3] Sampling period effect
[4] MUN: milk urea nitrogen

5.3.4. Dry matter intake and enteric CH_4 emissions

The effects of timing of paddock allocation on DMI and CH_4 emissions are shown in Table 4. Herbage DMI (*P* = 0.97) and total DMI (*P* = 0.9578) did not differ between AM and PM treatments. There was no treatment effect on daily enteric CH_4 emission (*P* = 0.9350), and efficiencies of milk (*P* = 0.6599), fat (*P* = 0.5750), protein (*P* = 0.3070), and milk solids (*P* = 0.6313) yield per g of CH_4 emitted. Additionally, timing of paddock allocation did not affect CH_4 yield (CH_4/kg DMI; *P* = 0.3380).

Table 4. Daily dry matter intake (DMI) (kg of DM/cow) and enteric CH_4 emissions of dairy cows grazing rotationally managed elephant grass cv. Cameroon with new paddocks allocated to dairy cows either in the morning (AM) or in the afternoon (PM) (n = 10)

Item	**Treatments**		**SEM**[1]	***P*-value**		
	AM	**PM**		**Trt**[2]	**Per**[3]	**Trt×Per**
Daily DMI						
Herbage	11.6	11.6	0.3862	0.97	0.27	0.002
Total	17.3	17.3	0.4494	0.9578	0.5276	0.0033
CH_4 emissions						
g/d	307.9	309.4	15.35	0.9350	0.2848	0.5405
g/kg of milk yield	18.8	18.3	1.48	0.6599	0.7323	0.4390
g/kg of fat yield	543.2	516.7	39.15	0.5750	0.9411	0.6236
g/kg of protein yield	585.7	543.8	39.98	0.3070	0.6489	0.2556
g/kg of milk solids yield	154.4	148.8	11.50	0.6313	0.9790	0.3510
g/kg of DMI	17.3	18.5	1.23	0.3380	0.1543	0.8623

[1] Standard error of the mean
[2] Treatment effect
[3] Sampling period effect

5.4. Discussion

Sward structure is defined as the distribution and arrangement of above-ground plant-part components (Laca and Lemaire, 2000). In tropical grasses, characteristics such as pre- and post-grazing SSH and leaf-to-stem ratio of the pre-grazing herbage mass play, along with herbage allowance, an important role in determining herbage intake and animal performance (Da Silva and Carvalho, 2005; Carvalho, 2013; Congio et al., 2018). In the present study, sward structure characteristics and herbage allowance were similar for both treatments, excluding their effects on other evaluated responses.

Diurnal variations in herbage chemical composition are directly related to NFC accumulation as result of the balance between leaf photosynthesis and plant transpiration (Griggs et al., 2005; Gregorini, 2012; Vibart et al., 2017). In the present study, DM concentration increased by 18% from AM to PM herbage. Orr et al. (1997) reported increases of 57.3% and 44.4% for perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), respectively. However, most literature reported increases from 14 up to 27% (Ciavarella et al., 2000; Delagarde et al., 2000; Trevaskis et al., 2001; Gregorini et al., 2008; Abrahamse et al., 2009; De Oliveira et al., 2014; Pulido et al., 2015; Vibart et al., 2017). Diurnal changes in temperature, solar radiation, and relative humidity, coupled with accumulation of photosynthates explain the DM concentration from the morning to the afternoon period (Gregorini et al., 2009).

Several studies described the pattern of NFC accumulation during the day, mostly on temperate swards (Lechtenberg et al., 1971; Orr et al., 1997; Ciavarella et al., 2000; Griggs et al., 2005; Gregorini et al., 2006; Shewmaker et al., 2006; Gregorini et al., 2008; Morin et al., 2011). Greatest concentrations of SC and starch in plants growing in temperate regions were reported between 12-13h after sunrise (Lechtenberg et al., 1971; Morin et al., 2011; Morin et al., 2012; De Oliveira et al., 2018). In our study, afternoon herbage samples were taken approximately 10 h after sunrise (4 pm), with increases of 52% in SC and 93% in starch for PM herbage compared to AM herbage. Greater increases of SC were found in tropical grasses (mean of 68%; Trevaskis et al., 2001; Fisher et al., 2005; De Oliveira et al., 2014). For temperate swards, including grass and legumes, Pelletier et al. (2010) reported increases of SC from 6 to 105% for PM herbage compared to AM herbage; however, most results reported mean increases of around 50% (Ciavarella et al., 2000; Mayland et al., 2000; Pelletier et al., 2010; Vasta et al., 2012; Pulido et al., 2015; Vibart et al., 2017). Increases in starch have been reported around 100% for PM temperate forage legumes (Orr et al., 1997; Brito et al., 2008; Pelletier et al., 2010; Andueza et al., 2012) and 30% for PM temperate forage grasses (Orr et al., 1997; Bertrand et al., 2008; Pelletier et al., 2010; Brito et al., 2016).

The increase in NFC and DM during the day dilutes other nutritional entities such as NDF, ADF and CP (Gregorini, 2012; Vibart et al., 2017). In the present study, PM herbage had decreased NDF (-2.9%) and ADF (-6.3%) relative to AM herbage but there was no effect in CP. Burns et al.

(2007) reported decrease of 7.4 and 6.7% for NDF and ADF, respectively, and no effect in CP concentration for PM alfalfa (*Medicago sativa* L.). Similar results were found in perennial ryegrass by Orr et al. (2001) and Abrahamse et al. (2009). Considering CP and N fractions, studies reported a decrease on PM compared to AM herbage (De Oliveira et al., 2014; Pulido et al., 2015; Vibart et al., 2017) while others showed no effect (Delagarde et al., 2000; Fisher et al., 2002; Gregorini et al., 2008). In fact, greater concentrations of SC and starch in the afternoon improve the NFC/PROT ratio which would optimize the supply of energy and protein to rumen microorganisms (Bryant et al., 2012; Bryant et al., 2014) reducing urinary-N excretion and losses onto pastures (Gregorini et al., 2010; Gregorini, 2012; Vibart et al., 2017).

The sampling period effect observed for some herbage chemical composition parameters might be explained by the post-grazing SSH. During P_2, post-grazing SSH was 3.6 cm lower than during P_1, which resulted in slightly greater proportion of stems on the pre-grazing herbage mass (1.9% for P_2 and 0.6% for P_1; $P = 0.037$). Stems contain greater proportion of cell wall and less photosynthetic tissues than leaves (Wilson and Kennedy, 1996) which explains the greater DM, NDF, NDICP, and lower digestible protein reported during the P_2. On the other hand, greatest lipid content in plants is found within the chloroplasts (Harwood, 1980), most present in leaves relative to stems.

Daily herbage intake was similar between treatments, which is in agreement with previously reported findings for grazing dairy cows (Gibb et al., 1998; Orr et al., 2001; Abrahamse et al., 2009; Mattiauda et al., 2013; Pulido et al., 2015; Vibart et al., 2017). On the other hand, studies that compared AM and PM herbage for housed ruminants reported greater DMI for animals fed with feedstuffs harvested at sundown (Fisher et al., 1999; Burns et al., 2007; Pagano et al., 2011; Andueza et al., 2012; Brito et al., 2008; 2009; 2016). Herbage DMI of grazing animals is a complex process strongly influenced by non-nutritional or behavioral factors such as sward structure and foraging behavior, whilst for housed animals herbage chemical composition and digestibility seem to be more relevant in setting DMI (Poppi et al., 1987; Hodgson, 1990; Da Silva and Carvalho, 2005; Carvalho, 2013).

Milk yield from dairy cows grazing new paddocks allocated either in the morning or in the afternoon showed only trends rather than significant treatment effects. Orr et al. (2001), although noticing a trend ($P = 0.076$) of 5% increase in milk yield for PM cows over 4 experimental weeks, concluded that there was no effect during the entire experimental period. Abrahamse et al. (2009) reported significant increase ($P < 0.05$) in fat and protein corrected milk yield and fat yield, even though no differences in milk yield were observed. Mattiauda et al. (2013), restricting grazing time to 4 hours of both periods of paddock allocation, observed a significant increase ($P < 0.05$) in protein yield for PM cows. Pulido et al. (2015) reported no differences in milk and components yields. Recently, Vibart et al. (2017) reported trends ($P < 0.10$) of greater fat, protein, and milk solids yield for PM cows. In this study trends for greater protein ($P = 0.0899$) and casein ($P = 0.0632$) yields were detected for cows grazing PM herbage. Brito et al. (2016) explained that greater proportion of supplements may dilute the effect of high NFC of PM herbage. In the present study, concentrate meals represented on average 33% of total DMI, greater than the amounts used by Vibart et al. (2017) (no concentrate), Orr et al. (2001) (22%), and Abrahamse et al. (2009) (17%), and lower than Mattiauda et al. (2013) (62%) and Pulido et al. (2015) (43%).

Indoor studies have shown that herbage intake of more balanced fermentable carbon to nitrogen ratio from PM herbage can improve N utilization of dairy cows (Brito et al., 2008; 2009; 2016). The authors reported lower N intake, urinary-N concentration, N excretion, and more N partitioning, with greater milk and protein yields for cows eating PM herbage. They also reported lower MUN for PM cows indicating that an improved balance in the supply of energy from NFC and N can reduce the excretion of urea N in milk. For grazing dairy cows, Vibart et al. (2017) observed trends of greater N use efficiency with moderate increases in N captured towards milk. In this experiment the higher NFC/PROT ratio in the PM herbage reduced the excretion of urea in milk and increased N into protein and casein yield. This simple and non-cost grazing management strategy can be an useful tool to improve N efficiency use of dairy cows and reduce N environment footprint in dairy farming systems.

Enteric CH_4 is influenced by the amount and nature of feed ingested by ruminants (Janssen, 2010). In this study, although timing of new paddock allocation has markedly affected herbage chemical composition, it did not affect DMI. Factors that increase passage rate (i.e. higher nutritive value and DMI) decrease CH_4 formation per unit of feed eaten (Blaxter and Clapperton, 1965; Janssen, 2010; Hammond et al., 2013). The model proposed by Janssen (2010) suggests that greater passage rates increase hydrogen concentration in the rumen making microorganisms select pathways that produce less hydrogen, resulting in less CH_4/kg of DM ingested. However, Hammond et al. (2013) reported that 0.85 and 0.87 of total variation in daily enteric CH_4 emissions of grazing sheep were predicted by DM and OM intakes, respectively; while herbage chemical composition showed weak correlation with both daily CH_4 emission and CH_4 yield (g/kg DMI). Although Ellis et al. (2012), in a modeling exercise, showed the possibility to reduce enteric CH_4 emission intensity of dairy cows fed with high-sugar grasses, it was not confirmed by the results from this field experiment.

5.5. Conclusions

The results ratify the general understanding of diurnal variation in herbage chemical composition towards greater concentrations of NFC and DM, and lower concentration of fiber components in the afternoon herbage. However, the increase in nutritive value of the afternoon relative to the morning herbage was not enough to increase DMI and milk yield, or to decrease CH_4 emission intensity by the dairy cows as hypothesized. The findings also indicate that new paddock allocation during the afternoon can be a simple and useful grazing strategy that results in greater N partitioning to protein yield, and lower excretion of urea N in milk.

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6. GENERAL CONSIDERATIONS

At the present time, the expected demand for food places farming systems under pressure (Chiavegato et al., 2018), but the increase in agricultural outputs has to be coupled with the decrease in environmental footprint (Godfray et al., 2010; Foley et al., 2011). In developing countries, agricultural production must increase 80% through higher yields resulting from intensification of existing agricultural systems (Conforti, 2011). In this sense, the concept of sustainable intensification began to be addressed in such systems as a means of achieving higher yields through practices that decrease the impact of key environmental issues (Royal Society, 2009; Garnett and Godfray, 2012).

Dairy farming systems from temperate pastures are more intensive than those from tropical pastures (Congio et al., 2018) and their intensification is usually associated with more inputs of nitrogen, to boost forage growth, or external supplementary feed, both aiming at increasing stocking rate and productivity (Ramsbottom et al., 2015; Macdonald et al., 2017). In the tropics, dairy farming systems besides having low N inputs, usually adopt inadequate grazing management strategies resulting in low levels of milk productivity. Therefore, the sustainable intensification of tropical pasture-based dairy systems may be possible through adoption of adequate grazing strategies rather than extra nitrogen inputs or additional supplementary feed (Congio et al., 2018), provided that minimum levels of soil fertility are provided to meet plant nutritional requirements.

This study was based in the literature that described the growth pattern of tropical forage grass species under grazing (Carnevalli et al., 2006; Barbosa et al., 2007; Trindade et al., 2007; Da Silva et al., 2009; Difante et al., 2009; Giacomoni et al., 2009; Barbosa et al., 2011; Gimenes et al., 2011; Zanini et al., 2012; Silveira et al., 2013; Geremia et al., 2014; Pereira et al., 2014; Pereira et al., 2015a; Pereira et al., 2015b; Silveira et al., 2016; Da Silva et al., 2017; Pereira et al., 2018; Sbrissia et al., 2018). In general, there is a change in plant growth and pattern of herbage accumulation during regrowth after reaching the canopy critical leaf area index (i.e. $LI_{95\%}$), when stem elongation and dead material accumulation increase at the expense of leaf accumulation. Further, it has been systematically observed that there is a positive relationship between canopy light interception and sward surface height (SSH), indicating that SSH may be used as a reliable field index for monitoring and controlling herbage regrowth (Da Silva et al., 2015). The results from this study corroborated the greater leaf accumulation, herbage nutritive value, greater grazing efficiency, better tussock distribution, and lower grazing losses on swards managed with the $LI_{95\%}$ relative to the LI_{Max} pre-grazing target. The results from this study also integrated animal with plant responses and showed that the pattern of plant growth during regrowth when managed with the $LI_{95\%}$ target provides benefits to grazing animals and to the system such as greater dry matter intake, higher milk yield and stocking rate resulting in 51% increase in milk productivity. Additionally, benefits regarding issues of environmental concern were also associated with this grazing management strategy, and corresponded to mitigation of emissions of the two most representative greenhouse gases (GHG) in dairy farming systems per kg of milk produced (i.e. CH_4 and N_2O/kg of milk).

Once the ideal pre-grazing management target was established during the first experiment, the second experiment aimed at seeking the possibility of refinement by studying the ideal time of the day to move animals to a new paddock. The results indicated that allocation of a new paddock at the right pre-grazing condition during the afternoon provides herbage with a more balanced NFC/PROT ratio to dairy cows, resulting in improved balance of protein and energy supply, and favoring increased N retention through enhanced milk protein yield and less N as milk urea nitrogen. The association of the $LI_{95\%}$ pre-grazing target and PM allocation could bring economic, productivity and environmental benefits towards sustainable intensification of tropical pasture-based systems. Both findings highlight the opportunity to improve the efficiency of tropical pasture-based dairy systems through practices that decrease the impact of key environmental issues, in accordance with the principles of sustainable intensification.

Recently, land-based research institutes have been concentrating efforts in assessing sources of GHG emissions in a broad range of agricultural systems around the world in order to generate field data that can support accurate carbon footprint reports (Muñoz et al., 2016; Nascimento et al., 2016; Luo et al., 2018; Pontes et al., 2018). Particularly, there are few data available regarding GHG emissions in tropical regions, where most studies estimate carbon footprint from agricultural systems using IPCC data (Intergovernmental Panel on Climate Change), with results that may be not so accurate (Lessa et a., 2014; Cunha et al., 2016). Therefore, *in loco* studies are mandatory to support accurate carbon footprint reports in tropical climate regions. Other aspect that impairs determination of the real carbon footprint from agricultural systems is that most efforts are towards measuring sources of GHG and few to evaluate carbon sequestration and storage in the soil. Perennial tropical pastures on moist-warm climate have an enormous potential to increase soil organic carbon and offset GHG emissions from livestock pasture-based systems (Braz et al., 2013; Abdalla et al., 2018). Abdalla et al. (2018) highlighted that C_4 grass species under high grazing intensities in moist-warm regions are more likely to increase soil organic carbon than C_4 under low grazing intensity. Therefore, further research should focus on the analysis of carbon sequestration and stock in the soil, to achieve a more accurate estimate of carbon balance and, therefore, to encourage mitigation strategies and programs by producers in association with companies and policy makers.

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7. CONCLUSIONS

Strategic grazing management represented by the $LI_{95\%}$ pre-grazing target associated with moderate severity of defoliation (50% of the pre-grazing sward surface height) is an environmentally friendly practice that improves the use efficiency of allocated resources through optimization of processes involving plant, ruminant and their interface, and enhances milk production efficiency of tropical pasture-based systems. In addition, daily allocation of herd to new paddock in the afternoon might increase N partitioning to protein yield, and decrease excretion of urea N in milk. The association of $LI_{95\%}$ pre-grazing target and afternoon allocation could bring economic, productive and environmental benefits towards sustainable intensification of tropical pasture-based systems.