

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

Grazing strategies, animal performance and environmental sustainability in
intensive pasture-based milk production systems

Camila Delveaux Araujo Batalha

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

Piracicaba
2018

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**Grazing strategies, animal performance and environmental sustainability in intensive
pasture-based milk production systems**

versão revisada de acordo com a resolução CoPGr 6018 de 2011

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To God, for the gift of live.

**To my parents, Ana Maria D. A. Batalha and Juacy de Souza Batalha, for support and
love.**

To my son, Diego Delveaux Costa. You are my sunshine.

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**Trust in the Lord with all your heart and lean not on your own understanding;
in all your ways submit to him, and he will make your paths straight.**

Proverbs 3:5-6

CONTENTS

RESUMO.....	8
ABSTRACT	9
1 INTRODUCTION	11
REFERENCES	13
2 STRATEGIC GRAZING MANAGEMENT: A TOOL TO IMPROVE GRAZING SYSTEM PRODUCTION AND PROFITABILITY	15
ABSTRACT	15
2.1 INTRODUCTION	15
2.2 MATERIALS AND METHODS	16
2.2.1 Treatments and grazing Management.....	17
2.2.2 Lactation and Ruminant Metabolism Trial.....	18
2.2.3 Chemical Analysis and Calculations	20
2.2.4 Statistical Analysis	21
2.3 RESULTS AND DISCUSSION	22
2.4 CONCLUSIONS.....	30
REFERENCES	31
3 EFFECTS OF Paddock Allocation Time on the Performance, Ruminant Variables and Nitrogen Utilization of Mid-Lactation Dairy Cows Grazing Rotationally Managed Elephant Grass	39
ABSTRACT	39
3.1 INTRODUCTION	39
3.2 MATERIALS AND METHODS	40
3.2.1 Treatments and grazing Management.....	41
3.2.2 Lactation and Ruminant Metabolism Trial.....	41
3.2.3 Chemical Analysis and Calculations	42
3.2.4 Statistical Analysis	43
3.3 RESULTS AND DISCUSSION	44
3.4 CONCLUSIONS.....	52
REFERENCES	52
4 CONCLUSIONS.....	59

RESUMO

Estratégias de pastejo, desempenho animal e sustentabilidade ambiental em sistemas intensivos de produção de leite à pasto

No Brasil, a produção animal em pastagens é reconhecida por ser uma atividade pouco competitiva frente a outras oportunidades de uso da terra. Embora sejam inúmeros os tipos de sistemas de produção de leite no Brasil, nota-se que a utilização de pastagens é característica comum. Assim, a identificação de práticas adequadas e eficientes de manejo do pastejo contribuirá com aumento da produção por vaca e por unidade de área, além de contribuir para a sustentabilidade do sistema. A baixa eficiência do uso dos recursos naturais tem classificado a pecuária nacional como uma importante fonte de poluição ambiental devido à emissão de poluentes, como gases de efeito estufa e excreção de nitrogênio. A adoção de técnicas de manejo de pastagens respeitando os limites fisiológicos da forrageira e aumentando sua digestibilidade, podem reduzir a produção de metano por quilo de leite produzido no ambiente e a excreção de nutrientes. Os objetivos desta tese foram investigar variáveis metabólicas e desempenho animal de vacas leiteiras em capim-elefante cv. cameroon submetido a estratégias de pastejo rotativo. Capítulo 1: Neste estudo foram avaliadas duas estratégias de manejo de pastejo: meta de pré-pastejo de 95% versus máxima interceptação luminosa (IL). Em sistemas intensivos de produção de leite à pasto, o manejo baseado no $IL_{95\%}$ permite que vacas tenham acesso a pastos com maior relação folha: colmo, menores perdas de forragem, resultando em uma forragem com melhor composição química. Os animais pastejando forragem com $IL_{95\%}$ tiveram maior consumo de matéria seca e energia, com maior produção de leite por vaca e taxa de lotação resultando em maior produção de leite por área. Além disso, a estratégia permite a diminuição das emissões de metano por consumo de energia líquida quando comparado a máxima IL. No entanto, a eficiência do uso de N não aumentou com essa prática de manejo. Capítulo 2: O objetivo do segundo estudo foi avaliar os efeitos do período de início pastejo (a.m. ou p.m.) na produção de leite, variáveis ruminais e eficiência de uso de N de vacas leiteiras no terço médio da lactação. Em sistemas intensivos de produção de leite à pasto, o pastejo de novos piquetes no período da tarde não teve efeito sobre o consumo de forragem e produção de leite de vacas no terço médio da lactação. No entanto, o maior teor de carboidratos não fibrosos da forragem ao final do dia possibilitou o aumento da síntese de proteína microbiana, redução do nitrogênio uréico no leite e apresentou tendência para aumento da produção de proteína e caseína do leite em comparação à vacas que iniciaram o pastejo no período da manhã. Ao longo dos estudos desta tese houve uma melhora no valor nutritivo da forragem adotando $IL_{95\%}$ e da forragem pastejada no período da tarde. Assim, o pastejo no período da tarde deve ser adotado juntamente com $IL_{95\%}$ como ajuste fino em sistemas intensivos de produção de leite à base de pasto.

Palavras-chave: pastagem tropical; vacas leiteiras; emissão de gases de efeito estufa; uso de nitrogênio

ABSTRACT

Grazing strategies, animal performance and environmental sustainability in intensive pasture-based milk production systems

In Brazilian livestock, and its diverse ways of production, the management of grazing animals is known as the lowest return on investment on land use opportunities. Nevertheless, among different types of milk production systems, it is noted that the use of pasture grazing is a common feature between them. Thus, to achieve profitability and maintain system sustainability, the identification of the most adequate and efficient pasture management practices, can maximize production per cow and production per unit area. The low efficiency of this method of production, also classifies the national livestock as the major source of environmental pollution due the emission of pollutants, such as greenhouse gases and nitrogen. The adoption of pasture management techniques respecting forage physiological limits and increasing digestibility of nutrients, can reduce the excretion of nutrients and the production of methane per kilo of milk produced on the environment. The objectives of this thesis were to investigate metabolic variables, greenhouse gas emissions and animal performance for dairy cows grazing elephant grass subjected to rotational stocking strategies. Chapter 1: In this study was to evaluate two strategies of grazing management: pre-grazing targets of 95% versus maximum canopy light interception (LI). In intensive pasture-based milk production systems, the management based on LI_{95%} allows lactating cows to have access to pastures with lower proportion of stems, with higher proportions of young leaves better chemical composition and perform an efficient grazing with lower forage losses. Therefore, the LI_{95%} pasture management strategy results in higher energy intake, higher milk production per cow, higher stocking rates of pasture and higher milk yield per area. Also, the strategy allows the decrease of methane emissions per net energy intake when comparing to management based on LI_{Max}. However, dietary N use efficiency did not increase with this management practice. Chapter 2: The objective of the second study was to evaluate the effects of paddock allocation time (a.m. vs. p.m.) on milk production, ruminal variables and efficiency of N use of mid-lactation dairy cows. In intensive pasture-based milk production systems, allocating cows on new paddocks on p.m. time has no effect on forage intake and milk production of grazing mid-lactation cows. However, the higher content of nonstructural carbohydrate of forage from p.m. pastures increases the yield of microbial protein, decreases milk urea nitrogen and tends to increase the yields of milk protein and milk casein compared to a.m. pastures. Throughout this thesis there were an improvement on nutritive value of forage adopting LI_{95%} as a pre- grazing target and forage grazed at p.m. Therefore, the time of allocation on paddock should be used along with LI_{95%} as fine-tune in intensive pasture-based milk production systems.

Keywords: Tropical grass; Dairy cows; Greenhouse gas emissions; Nitrogen use

1 INTRODUCTION

Brazil is the fourth largest producer of milk worldwide. The major milk production comes from small farms with low pasture-based system productivity. Among the reasons the use of poor managed pasture highlights the extractive character of these producers. The low efficiency of this method of production, also classifies the national livestock as the major source of environmental pollution, such as greenhouse gases and nitrogen.

In intensive pastures-based milk production systems one of the limiting factor for high productivity is the maximization of forage intake. Animals' harvesting capacity is a complex mechanism affected by climate (e.g. temperature), sward structure and behavioral limitations. According to Poppi et al. (1987), forage intake, is related to sward structure and grazing behavior, and in a second moment is related to nutritional factors such as forage chemical composition, digesta retention time in the rumen and concentration of metabolic compounds. Grazing management strategies that prioritize leaves rather than stems and dead material should optimize the harvesting of better quality forage (Da Silva and Carvalho, 2005).

Recommended grazing management practices, such as the use of high doses of N fertilization (Johnson et al., 2001; Pereira et al., 2015) and adoption of a rotational grazing regime based on the physiological aspects of plants instead of the use of fixed grazing intervals have considerably improved the nutritive value of pastures, therefore, the forage intake (Palhamo et al., 2007). It has been reported in studies with pastures under rotational grazing that the interruption of regrowth when forage canopy reaches 95% of light interception results in a shorter defoliation interval, with greater leaf proportion and low accumulation of dead material, leading to a higher leaf: stem ratio in the sward composition (Pedreira et al., 2007). Consequently, a larger amount of living tissue of high nutritional value is available for the grazing animal (Voltolini et al., 2010; Pereira et al., 2013; Da Silva et al., 2017).

Besides the canopy structure the allocation time of animal on a new paddock can improve the nutritive value of forage intake (Delagarde et al., 2000; Gregorini et al., 2006; Brito et al., 2008; 2014; Vibart et al., 2017). The accumulation of carbohydrates by the photosynthetic process, continues throughout the day increasing the concentration of sugars in plant tissues, reaching a plateau. In the absence of light, the process is downregulated decreasing the concentration of carbohydrates and sugar (Curtis, 1944; Lechtenberg et al. 1971) in plant tissues. By the effect of light presence, forage grazed at sunset may alter nutrient supply when compared to plant grazed at sunrise (Oliveira et al., 2014; Vibart et al., 2017), increasing DMI (Brito et al., 2008; 2016), digestibility of organic matter (Brito et al., 2008, 2014) and milk yield

(Brito et al., 2008). Moreover, forages with higher total nonstructural carbohydrates can alter the ruminal fermentation and optimize the use of nitrogen (Brito et al., 2008).

Several studies have reported the benefits of grazing management strategies, most focused solely on plant responses, however there is a need relating plant and animal responses and environmental benefits in tropical pasture-based dairy systems. A conceptual model was created aiming at integrating the relationships among plant and animal as a function of grazing strategies in tropical pasture-based dairy systems (Figure 1).

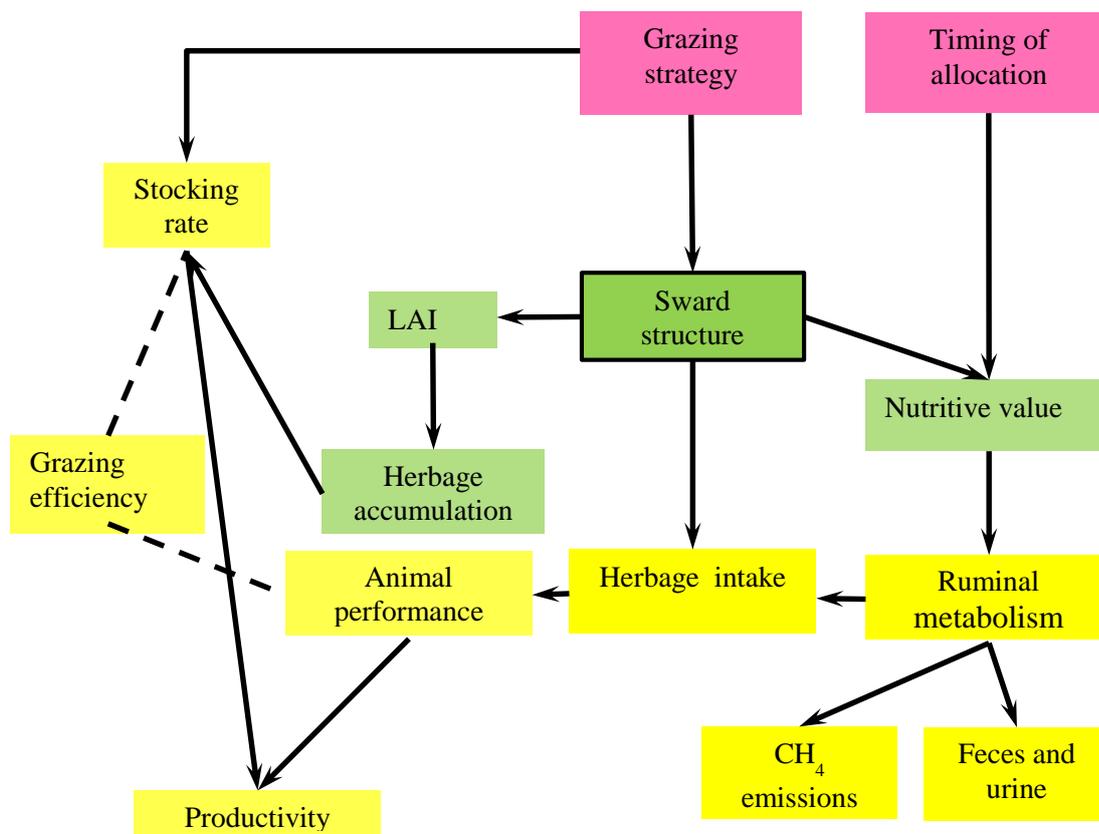


Figure 1- Conceptual model - Pink boxes: controlled factors (treatments); green boxes: plant responses; yellow boxes: animal responses.

Two experiments were conducted, in the first one, investigated the effects of two grazing management methods (pre-grazing targets of 95% and maximum canopy light interception) on sward structure, forage composition, cow performance, ruminal metabolism, nutrient digestibility, CH₄ emission and nitrogen use in dairy cows. Once the ideal pre-grazing target (LI95% or LIMax) was established during the first study, the second step consisted of a refinement of the first phase. The second study investigated the effects of was that paddock allocation at p.m. compared with a.m., would improve N utilization, DMI, and milk yield in

mid-lactation dairy cows grazing tropical grass. The central hypothesis of these thesis was that 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. The objectives of this thesis were to investigate metabolic variables, greenhouse gas emissions and animal performance for dairy cows grazing elephant grass subjected to rotational stocking strategies.

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2 STRATEGIC GRAZING MANAGEMENT: A TOOL TO IMPROVE GRAZING SYSTEM PRODUCTION AND PROFITABILITY

Abstract

The objective of this study was to evaluate two strategies of grazing management of a tropical grass on sward structure, metabolic variables, methane production, total tract neutral detergent fiber digestibility (TTNDF), performance, milk composition and nitrogen use in grazing dairy cows. Treatments corresponded to management strategies of elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) characterized by the pre-grazing targets of 95% versus maximum canopy light interception (LI_{95%} and LI_{Max}, equivalent to 100 and 135 cm, respectively). The post-grazing height targeted corresponded to 50% of the pre-grazing height. The pasture was fertilized with N according to the rest period, so that at the end of the experiment the two treatments received the same amount of fertilizer. Twenty-six mid-lactation multiparous crossbred (Holstein × Jersey) cows were used in a randomized block design with 3 periods of 40 d (last 7 d used for data and sample collection). The LI_{95%} treatment had sward structure characterized by more leaves, less stems, less dead material and lower losses. The LI_{95%} had a higher concentration of crude protein (CP), and lower contents of acid detergent fiber (ADF) and lignin. Dry matter intake (DMI) and nutrients digestibility was higher for cows subjected to LI_{95%} as well as predict TTNDF and ruminal potential digestible NDF fraction (pdNDF). Milk and milk protein yields were increased by LI_{95%}. Despite a higher urinary nitrogen excretion and milk urea N (MUN) for the latter treatment, the efficiency of N use was not affected. The rumen concentration of volatile fatty acids (VFA) was higher for LI_{95%}, with more butyrate and less acetate, leading to less emission of enteric CH₄ per unit of feed fermented. The improved sward structure and chemical composition of pastures managed with LI_{95%} resulted in higher milk yield and lower methane emission per Mcal of consumed energy, indicating this criterion as adequate for pasture management in intensive rotational systems.

Keywords: Enteric methane emission; Light interception; Nitrogen metabolism; Tropical pasture

2.1 Introduction

The possibility of using pastures for most parts of the year is one of the benefits of livestock production in the tropics, which is further benefitted from the low production costs in comparison to confined systems. However, this practice is usually associated with lower productivity in contrast with the latter system (Stott and Gourley, 2016). Ruminants have the capacity to digest low quality forages (Conrad et al., 1964; Allen, 2000), but the low productivity of tropical grasses can be attributed to poor practices of pasture management, which lead to excessive proportions of stems and dead material that reduce their harvesting efficiency and nutritive value.

Recommended grazing management practices, such as the use of high doses of N fertilization (Johnson et al., 2001; Pereira et al., 2015) and adoption of a rotational grazing regime based on the physiological aspects of plants instead of the use of fixed grazing intervals have considerably improved the nutritive value of pastures, therefore, the forage intake

(Palhamo et al., 2007). It has been reported in studies with pastures under rotational grazing that the interruption of regrowth when forage canopy reaches 95% of light interception (**LI**) results in a shorter defoliation interval, with greater leaf proportion and low accumulation of dead material, leading to a higher leaf: stem ratio in the sward composition (Pedreira et al., 2007). Consequently, a larger amount of living tissue of high nutritional value is available for the grazing animal (Voltolini et al., 2010; Pereira et al., 2013; Da Silva et al., 2017). However, there are few studies that relate directly the effects of grazing management of tropical grasses on milk production and milk composition, efficiency of N utilization (ENU) and ruminal metabolism in dairy cows.

The intensive management of tropical grasses can result in high fiber digestibility (Lopes, 2011) and CP content (De Souza et al., 2017), especially if higher doses of N fertilization are in place (Johnson et al., 2001). The high CP supplied by the base dietary forage has become the 'limitation' related to low ENU by dairy cows (Gregorini et al., 2016), causing a waste of around 75% of the N (Huhtanen and Hristov, 2009). However, the association of more digestible forages with the high CP content allows the use of supplements with lower protein content in the pasture-based system (Danés et al., 2013). Additionally, this is also associated with the reduction of enteric methane emission and system profitability (Doole, 2014).

According to Aguirre-Villegas et al. (2017), methane production is the main contributor to greenhouse gas emissions in dairy farms. Pasture-based systems have been charged as the main methane emitters in comparison to feedlot systems (De Léis et. al, 2015). The low quality pastures can be categorized as one of the main aspects responsible for the higher production of methane per kg of milk produced in grazing systems (Belflower et al., 2012). The use of forage types with lower fiber content and higher levels of soluble carbohydrates, or even grazing on early vegetative pastures, were identified as alternatives for the reduction of enteric CH₄ in pasture-based production systems (Beauchemin et al., 2008).

Therefore, the aim of this study was to evaluate different grazing strategies of a tropical grass on sward structure, forage composition, cow performance, ruminal metabolism, nutrient digestibilities, CH₄ emission and nitrogen use in dairy cows.

2.2 Materials and methods

The study was conducted in Piracicaba, Sao Paulo, Brazil (22°42'S, 47°38'W and 546 a.s.l.) from December, 2015 to April, 2016, on a rainfed, non-irrigated elephant grass

(*Pennisetum purpureum* Schum. cv. Cameroon) pasture established in 1972 in a high fertility Eutroferic Red Nitossol (Pereira et al., 2014). 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 25.9 °C and 169 mm respectively. All procedures involving the animals were approved by the Ethical Committee for Animal Research of the University of Sao Paulo, Piracicaba (protocol: 17.5.999.11.9).

2.2.1 Treatments and grazing Management

Treatments were allocated to experimental units (2058 m² paddocks) with elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) according to a randomized complete block design, with six replications. The slope and chemical soil characteristics were considered as blocking criteria. Each paddock was divided up into three sub-paddocks (686m²) with forage measurements performed within the central sub-paddock (Figure 1). Treatments corresponded to two grazing management strategies characterized by the pre-grazing sward height correlated to 95% (**LI_{95%}**) and to maximum canopy (**LI_{Max}**) during regrowth (equivalent to 100 and 135 cm of sward height, respectively). The post-grazing sward height corresponded to 50% of the pre-grazing height. The LI values and corresponding heights of the pasture were determined in the summer before the experiment. The LI was monitored using a LAI 2000 canopy analyser (LI-COR, Lincoln, NE, USA). The pasture was fertilized with N according to the rest period, so that at the end of the experiment the two treatments received the same amount of fertilizer (215 kg N/ha as urea). All pasture measurements were taken every day before the experimental animals entered the paddock and after they left. Sward height was taken in 40 randomized points in each measurement. Pre-grazing forage mass and morphological composition were determined in 2 representative points selected in the paddock, and in each point, the material within a square frame (1.25 x 0.75 m) was cut at the post-grazing height targeted for each treatment. Pre-grazing forage mass was weighed, and a representative subsample was taken for determination of leaf blades, stems (including leaf sheaths), and dead material (as indicated by more than 50% of the tissue area being senescent) to determine the sward morphological composition. The forage grazing losses were measured in 2 representative points of average sward condition with a square frame (1.25 x 0.75 m) allocated before the animals entered the paddock and the losses were measured according to Carnevalli et al. (2006) after they left. Pre- and post-grazing sward height, pre-grazing forage mass, grazing losses and morphological composition are presented in Table 1. Hand-plucked forage samples were also collected before

the animals entered the paddock. The samples were plucked between the thumb and a backward-bent forefinger, simulating the cows' grazing behavior as close as possible, by the same person over the entire experiment (De Vries, 1995). Pasture samples were dried in a forced-air oven at 65°C for 72 h, ground through a 1mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA) and stored at room temperature for further analysis.

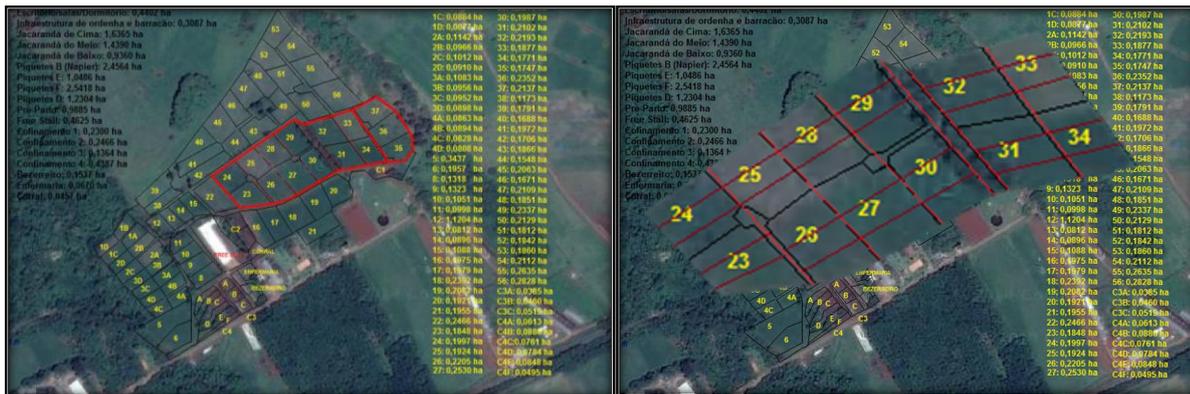


Figure 1 - Experimental units (2058 m² paddocks, 23 to 34) with elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) with six replications, and the sub-paddocks (686m²).

2.2.2 Lactation and Ruminal Metabolism Trial

Twenty-six mid-lactation crossbred (Holstein × Jersey) cows were used, averaging (mean ± SD) 126 ± 90 d in milk (DIM), 20.3 ± 2.6 kg/d of milk and 488 ± 60 kg of body weight (BW) at the beginning of the trial. All cows received the same amount of concentrate supplement for a preliminary period of 15 d, and then were blocked based on milk yield, DIM and BW. Cows grazed the pastures as a single group per treatment (13 cows per treatment group). The experiment lasted from December 2015 to April 2016. For the metabolism trial, the experimental period was divided in 3 periods of 40 d with the last 7 d of each period for data and sample collection. 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 considering the number of cows (experimental and additional cows) used daily for each treatment.

Concentrate was fed individually twice a day at 4:30 a.m. and 2:30 p.m., before each milking, at a rate of 1 kg of concentrate (as fed) / 3 kg of milk, based on milk yield at the end of the preliminary period and again at the end of each period for each block. The same amount

of concentrate was fed for cows of both treatments within block. The concentrate was composed of citrus pulp (35%), corn gluten feed (30%), ground corn (20%), soybean meal (10%) and minerals (5%), resulting in 14% of CP (on DM base). Samples of concentrate were collected in 5 consecutive days at the end of each metabolism experimental period, bulked by period and stored at room temperature for further chemical analysis.

Milk yield was measured on a daily basis and milk composition at 10 d intervals during the 120 days' experimental period. Milk samples from both milking were stored in vials with a bronopol preservative pill and sent immediately for composition analysis. Cows were weighed at the end of each metabolism experimental period during 3 consecutive d after the morning milking. Body condition scored (BCS) was also assessed at the same day, by 2 trained investigators on a 5-point scale, where 1 = thin and 5 = fat, as described by Wildman et al. (1982).

Forage intake and *in vivo* diet nutrient digestibility were measured at the end of each metabolism sampling period based on total fecal excretion and feed indigestibility. To determine fecal excretion, TiO_2 was orally dosed twice a day (20 g/cow per d) after concentrate meals. Fecal grab samples were collected after morning and afternoon milking on the last 5 d of each period, and stored in a -20°C freezer. Subsequently, samples were thawed at room temperature, bulked by cow, dried at 55°C in a forced-air oven, and ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA). To calculate *in vivo* feed digestibility, the internal marker indigestible neutral detergent fiber (iNDF) was estimated using an *in vitro* incubation in buffered rumen fluid for 240 h (Goesser and Combs, 2009) of fecal samples from individual cows and feed composite samples (concentrate and hand-plucked pasture samples).

An *in vitro* assay was also conducted to estimate total tract neutral detergent fiber digestibility (TTNDFD), rumen potentially digestible NDF (pdNDF) and pdNDF degradation rate (kd) of forage using a model described by Combs (2013).

In order to estimate microbial synthesis, the purine derivative excretion method of Chen and Gomes (1992) was used. Spot samples of urine were collected 4 h after the morning concentrate feeding on the last d of each period. Ten milliliters of urine were mixed with 40 mL of 0.072 N of H_2SO_4 and stored at -20°C .

Enteric methane production was determined in each period, using the sulfur hexafluoride (SF_6) tracer technique (Johnson and Johnson, 1995). On the first day of the trial each cow was intraruminally dosed with a calibrated permeation tube of SF_6 (1.41 ± 0.401 mg/d), which remained in the rumen throughout the experiment. Representative breath samples

of each animal were collected in a pre-evacuated canister (~2.3 L) by means of capillary tubing fitted to a halter. The canisters were changed every 24 h before the afternoon milking. At the same time, background air gas samples were collected once daily in the barn and near the paddocks. Daily gas samples (breath, background) were taken over 7 d. At the end of each period, they were immediately transported to the laboratory for SF₆ and CH₄ analyses.

Rumen fluid was sampled through esophageal probe from all cows at the end of each period 4 h after the morning concentrate feeding. A sample of 50 mL of ruminal fluid was filtered and immediately stored at -20 °C for volatile fatty acid (VFA) determination.

2.2.3 Chemical Analysis and Calculations

Hand-plucked pasture, concentrate, and fecal samples were analyzed for DM (drying samples in an oven at 105°C for 24 h), ash (AOAC International, 2005; method 942.05), total N content (Leco FP-2000 N Analyzer; Leco Instruments Inc., St. Joseph, MI), ether extract (method 920.85; AOAC, 1986), NDF (Van Soest et al., 1991) using sodium sulfite for all samples and heat-stable α -amylase for concentrate samples. Feed samples were also analyzed for ADF and lignin (AOAC International, 2005; method 973.18).

Hand-plucked pasture samples were also analyzed for neutral detergent insoluble protein (NDIP), acid detergent insoluble protein (ADIP) without Na₂SO₃ (Van Soest et al., 1991) and nonprotein nitrogen (NPN) (Licitra et al., 1996) to quantify the CNCPS protein fractions: A+B1 (soluble NPN and soluble true protein, respectively), B2 (protein with intermediate rates of degradation), B3 (CP insoluble in neutral detergent solution but soluble in acid detergent solution) and C (unavailable N) (Sniffen et al., 1992).

Forage intake was calculated from total fecal excretion and feed indigestibility. To determine fecal excretion, fecal samples were analyzed for titanium concentration according to Myers et al. (2004).

Feed and fecal samples were analyzed for indigestible NDF (**iNDF**; NDF remaining after 240 h *in vitro* incubation). Total fecal excretion, fecal excretion coming from the concentrate and forage intake were calculated according to De Souza et al. (2015). For *in vitro* TTNDFD prediction the pdNDF was calculated from the difference of total NDF and iNDF (pdNDF = NDF - iNDF; NRC, 2001), kd of pdNDF is calculated from NDF residue measurements taken at 24, 30, and 48 h of *in vitro* incubation in rumen fluid (Goeser and Combs, 2009) using a first-order kinetics model with an indigestible fraction as described by

Mertens (1993) and TTNDF digestibility is expressed as a percentage of total NDF as TTNDFD = $100 \times \{pdNDF \times [kd / (kd + kp)]\} / 0.9$.

Milk samples were analyzed for fat, protein, lactose, total solids (TS), and MUN using infrared procedures (Foss 4000; Foss North America, Eden Prairie, MN).

NE_L intake and was calculated from DE according to NRC (2001). Energy values were calculated according to NRC (2001) as follows: Net energy (NE_L) intake = DMI . NE_L(diet); Milk NEL (Mcal/ d) = milk yield (kg). [0.0929 . (fat %) + 0.0563 . (true protein %) + 0.0395 . (lactose %)]; NEL BW gain = BW change . 5.65; NE_L activity = (distance between the milking center and pasture . 0.00045 . BW) + (0.0012 . BW); NE_L available maintenance = NEL (intake) – NEL (BW gain) – NEL (milk) – NEL (activity).

Apparent efficiency of N use (ENU; assuming no retention or mobilization of body N) was calculated for each cow by dividing mean milk N output (milk CP/ 6.38) by total N intake. Urinary N excretion was calculated from total urine production times its N content. Urine samples were thawed and analyzed for creatinine, allantoin, and uric acid using HPLC. Total urine production was estimated by creatinine content, assuming that the excretion of creatinine was constant at 0.213 mmol/kg body weight (BW; Chizzotti et al. 2008).

For methane analysis, gas concentration (SF₆ and CH₄) in breath and ambient air was determined using a gas chromatograph HP6890 (Agilent, Newport, DE, EUA) fitted with an electron capture detector (ECD, for determination of SF₆) and flame ionization detector (FID, for determination of CH₄). Ruminal fluid samples were thawed at room temperature and centrifuged (15,000 × g, 4°C, 30 min), and the supernatant was analyzed for VFA by gas chromatography (Palmquist and Conrad, 1971).

2.2.4 Statistical Analysis

The data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). Within forage parameters, the paddock was considered the experimental unit, and for animal measurements, the cow was considered the experimental unit. All variables were analyzed as repeated measures. The statistical model used was:

$$Y_{ijk} = \mu + B_i + C_j + T_k + CT_{jk} + e_{ijk}$$

Where Y_{ijkl} is the variable of interest, μ is the overall mean, B_i is the random effect of block (i = 1 to 13 for animals and 1 to 6 for forage), C_j is the fixed effect of treatment (j= LI_{95%} and

LI_{Max}), Tk is the fixed effect of time (k = period), CT_{jk} is the fixed effect of interaction between treatment and time, and e_{ijk} is the residual error. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Data were considered outliers and excluded from the data set when a Student's residual higher than 2 or lower than -2 was detected. Denominator degrees of freedom were adjusted by the Kenward-Rogers method. Significances was determined at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

2.3 Results and discussion

The pasture characteristics is presented in Table 1. The average sward heights reached throughout the experimental periods were the following: pre-grazing 99.7 and 134.4 cm, post-grazing 50.9 and 68.3 cm for treatments LI_{95%} and LI_{Max}, respectively. All the forage mass data collected refers to measurements above the post-grazing height. The LI_{Max} had more forage mass (2890 vs 4890 kg of DM/ha; $P < 0.001$), however 16.6% represented by stem whereas LI_{95%} had less mass with more leaves and less stems (95.1 and 3.5%, respectively). Thus the leaf: stem ratio was significantly higher for LI_{95%} (32.8 vs 4.6% of forage DM; $P < 0.001$). Dead material was not different between treatments (1.9% of forage DM; $P = 0.31$), probably because the sampling was performed above the residue height. The morphological differences are shown in Figure 2, where it is possible to observe a large accumulation of dead material below the height of the residue. The LI_{Max} had more losses (20.1 vs 10.7% of forage DM; $P < 0.001$). These results agree with prior studies where forages managed based on LI_{95%} criterion presented significantly higher proportion of leaves and lower proportion of stem and dead material in comparison to forages managed with LI_{Max} or fixed grazing intervals (Carnevalli et al., 2006; Voltolini et al., 2010; Pereira et al., 2014).



Figura 2- Grazing management strategies characterized by the pre-grazing sward height correlated to 95% and to maximum canopy during regrowth (equivalent to 100 and 135 cm of sward height, respectively).

Well-managed pastures provide improvements on system profitability, because it is linearly related to pasture utilization per hectare (Ramsbottom et al., 2015). The forage losses were 289 kg of DM for the LI_{95%} compared to 978 kg/ha for the LI_{Max} strategy. In a companion paper (Congio et al., 2018) LI_{95%} allowed more grazing cycles (5.6 vs 3.5) with less forage losses and an increase in the stocking rate (9.3 vs 7.0 cows/ha).

Table 1. Characteristics at pre- and post-grazing of elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) pasture submitted to rotational grazing management strategies

Characteristic ¹	Treatment (Trt) ²		SEM	P-value		
	LI _{95%}	LI _{Max}		Trt	Time	Trt*time
Sward height pre-grazing, cm	99.7	134.4	0.64	-	-	-
Sward height pos-grazing, cm	50.9	68.3	0.47	-	-	-
Forage mass, kg of DM/ha	2890	4890	208	<0.001	0.36	0.34
Leaves mass, kg of DM/ha	2747	3924	147.1	<0.001	0.11	0.07
Morphological composition, % of forage DM						
Leaves	95.1	81.1	0.93	<0.001	0.76	0.10
Stem	3.5	16.6	0.52	<0.001	0.09	0.20
Dead material	1.4	2.3	0.2	0.31	0.01	0.45
Losses	10.7	20.1	1.67	<0.001	<0.001	0.78
Leaf: stem ratio	32.8	4.6	3.5	<0.001	<0.001	<0.001
Density, kg of DM/cm	59.6	79.7	3.63	<0.001	0.96	0.82

¹All measurements were taken above the post-grazing residue (50% of the pre-grazing height), except for pre and pos-grazing heights.

²LI_{95%} = 95% canopy light interception and LI_{Max} = Maximum canopy light interception.

The chemical composition of elephant grass is presented in Table 2. To achieve maximum LI, the sward needed more time (31.7 vs 21.1 d of grazing interval) and had more participation of stems than LI_{95%}, thus LI_{Max} presented a higher concentration of ADF (36.3 vs 33.9% of DM, $P = 0.03$), lignin (3.8 vs 3.3% of DM, $P = 0.004$) and lower concentration of CP (19.4 vs 21.0%, $P < 0.001$). In lower sward heights occur an intense tissue turnover that promotes the appearance of young leaves, which are more photosynthetically active than old leaves, commonly found in LI_{Max} (Da Silva et al., 2013). In addition, tall swards require greater deposition of sustaining tissue which are mainly composed of vascular bundles and sclerenchyma, present low nitrogen content and high fiber values (Palhano et al., 2007). Other parameters of the chemical composition evaluated in this study were not affected by the treatments ($P > 0.05$). Despite the significant difference between the forage CP contents (Table 2), most of the CP had intermediate or low rates of degradation for both treatments, with no differences on CP fractions between the treatments ($P > 0.05$). These results agree with Juarez Lagunes et al. (1999) that evaluated 15 tropical grasses according to Cornell Net Carbohydrate and Protein System (CNCPS), and in all forages the CP was mainly represented by NDIP (% of CP).

Table 2. Chemical composition of hand plucked samples of elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) pasture submitted to strategies of rotational grazing management

Item, (%)	Treatment (Trt) ¹		SEM	P-value		
	LI _{95%}	LI _{max}		Trt	Time	Trt*time
DM	19.5	19.2	0.91	0.70	0.07	0.28
Organic matter	89.6	88.8	0.54	0.28	0.02	0.68
Ash	10.7	11.2	0.54	0.28	0.02	0.68
CP	21.0	19.4	0.50	<0.001	<0.001	0.04
A+B1 ²	24.3	23.1	1.38	0.55	<0.001	0.005
B2 ²	39.8	39.7	1.61	0.94	<0.001	<0.001
B3 ²	31.0	32.1	1.03	0.46	0.08	0.73
C ²	5.4	6.0	0.59	0.54	0.04	0.30
NDF	61.2	63.0	1.26	0.11	0.14	0.29
ADF	33.9	36.3	1.14	0.03	0.26	0.74
Lignin	3.3	3.8	0.16	0.004	0.24	0.47
Ether extract	3.1	3.2	0.19	0.68	0.001	0.12
NFC ³	3.9	3.2	0.8	0.34	0.76	0.77

¹LI_{95%} = 95% canopy light interception and LI_{Max} = Maximum canopy light interception.

²Fraction of crude protein (% of CP; Sniffen et al., 1992).

³Nonfiber carbohydrates = 100 – (NDF + ash + CP + ether extract).

Nutrients intake and apparent total tract digestibility are presented in Table 3. According to predictions of NRC (2001) and Dórea et al. (2017) to maintain the milk yield difference of 2.3 kg/d, animals of the LI_{95%} treatment should consume 1.0 kg and 1.3 kg/d of diet DM more than animals of LI_{Max} treatment, respectively. The DMI predicted by the NRC (2001) were 16.3 and 15.3 kg/d, while the values predicted with the external marker were 18.2 and 15.9 kg/d for LI_{95%} and LI_{Max}, respectively. The use of markers to predict DMI may present some problems resulted from a faster passage through the rumen in comparison to fibrous material (Van Soest, 1994), and/or variations in excretion throughout the day (Smith e Reid, 1955) and incomplete recovery of the marker in the feces. The sward structure and chemical composition of LI_{95%} allowed a higher DMI, with 2.2 kg/d of forage DMI above the value observed in LI_{Max} (12.3 vs 10.1; $P = 0.002$). The higher proportion of stems in LI_{Max} can result in a physical constraints reducing forage intake (Laca and Lemaire, 2000) and the higher ADF and lignin concentration can result in increase of retention time in rumen-reticulum causing distention and limiting DMI (Allen, 2000). Is likely that the combination of higher leaf: stem ratio for LI_{95%} with greater digestibility of nutrients have allowed higher harvest efficiency and less rumen fill, resulting in greater forage intake. These results are in agreement with other studies where higher DMI and NDF digestibility were reported for LI_{95%} (Da Silva and Crvalho, 2005; Paula et al. 2012; Munõs et al., 2016).

The difference in amount of forage lost during the grazing process between both management strategies was 689 kg of DM/ha per grazing cycle (978 vs 289 kg/ha). This expressive reduction in forage losses with the LI_{95%} strategy allows greater pasture stocking rate (Congio et al., 2018) with cows consuming more forage (Table 3) and producing more milk (Table 4).

No difference was found on *in vitro* digestion rate of forage (Table 3). this result may be due the preparation of the samples for *in vitro* assay, where the samples were ground through a 1-mm screen. The LI_{95%} had higher forage ruminal pdNDF (76 vs 70% of total NDF; $P < 0.01$) and TTNDFD (49.1 vs 43.2% of total NDF; $P = 0.015$). Characterization of tropical grass species by fiber digestibility, especially in the case of intensively managed grass, is scarce in the literature. Lopes (2011) evaluated the nutritive value and fiber digestibility of the main tropical grasses produced under intensive rotational grazing managements in Brazil, and reported 44.6% on NDF basis for *in vitro* digestibility of elephant grass (time points: 24, 36 and 48 h). Assuming 10% of hindgut digestion of NDF (Lopes et al., 2015) the TTNDFD was 49.5% of NDF, in agreement with the present study for LI_{95%}. A lower digestibility of the LI_{Max} forage was most likely due to a combination of higher fiber content (Table 2) with fiber of lower digestibility (Table 3). The decrease in fiber quality may be explained by the higher content of lignin and ADF, as a result of the increased maturity due to the longer time required to reach maximum LI. Fiber concentration increases as plants mature, especially in stems, contributing to the depression of forage digestibility (Buxton and Redfearn, 1997; Moore and Jung, 2001). Krueger et al. (2008) described that the digestibility of C4 grasses is mainly affected by cross linkages and high lignin concentrations, which restrict the degradation of digestible xylans by ruminal microorganisms.

Table 3. Effects of strategies of rotational grazing management on nutrients intake and digestibility of dairy cows

Item	Treatment (Trt) ¹		SEM	P-value		
	LI _{95%}	LI _{Max}		Trt	Time	Trt*time
Intake, kg/d						
Total DMI	18.2	15.9	0.61	0.003	0.11	0.22
Forage DMI	12.3	10.1	0.52	0.002	0.01	0.18
OM	16.2	13.5	0.55	<0.001	0.68	0.11
CP	3.3	2.7	0.12	<0.001	0.01	0.88
NDF	8.6	7.6	0.35	0.043	0.03	0.11
Digestibility ² , %						
DM	62.9	57.8	0.64	<0.001	0.23	0.27
Organic matter	67.13	60.57	0.81	<0.001	0.10	0.12
CP	69.9	65.2	0.86	<0.001	<0.001	0.11
NDF	56.4	50.0	0.90	<0.001	0.11	0.89
TTNDFD ³ , % of total NDF	49.1	43.2	1.5	0.015	0.98	0.08
pdNDF ⁴ , % of total NDF	76	70	0.8	<0.001	0.44	0.01
pdNDF kd ⁵ , %/h	3.6	3.4	0.30	0.63	0.69	0.99

¹LI_{95%}: 95% canopy light interception and LI_{Max}: Maximum canopy light interception.

²*In vivo* digestibility

³TTNDF: predicted total-tract NDF digestibility using *in vitro* TTNDFD model.

⁴pdNDF: potential digestible NDF calculate from TTNDFD model.

⁵pdNDF kd: potential digestible NDF fraction digestion rate calculate from TTNDFD model.

Milk yield and composition are presented in Table 4. Milk yield was 2.3 kg/d higher for LI_{95%} (18.05 vs 15.74, $P < .0001$), with lower concentration of milk protein (3.12 vs 3.35%, $P = 0.001$), but with greater protein yield per day (0.56 vs 0.52 kg/d, $P = 0.01$). In the companion paper (Congio et al., 2018) the adoption of the LI_{95%} strategy increased milk production per area in 57 kg/ha/d (169.8 vs 112.4 kg/ha/d, $P = 0.0012$). Voltolini et al. (2010) reported also higher milk production, higher stocking rates (7.2 vs 5.1 cows/ha) and consequently higher productivity (114 vs 75 kg/ha/d) when cows grazed elephant grass cv. Cameroon managed using 95% LI (103 cm canopy height) as the pre-grazing height in comparison with cows grazing paddocks managed with 27 d of fixed grazing intervals. The higher DMI with higher nutrients digestibility increased the NE_L intake for LI_{95%} (25.7 vs 20.8 Mcal/d; $P < 0.001$; Table 6), allowing a higher milk production and milk NE_L (12.3 vs 11.2 Mcal; $P = 0.03$). However, due the higher DMI, the efficiency (Milk NE_L / NE_L intake) was lower for this treatment (47.4 vs 53.8 %; $P = 0.006$). No differences in BCS change was observed ($P > 0.05$).

Table 4. Effects of strategies of rotational grazing management on milk yield and composition

Item	Treatment (Trt) ²		SEM	<i>P</i> -value		
	LI _{95%}	LI _{Max}		Trt	Time	Trt*time
Milk, kg/d	18.05	15.74	1.01	<0.001	<0.001	0.78
3.5% FCM ¹ , kg/d	18.40	16.88	1.18	0.02	0.02	0.63
Fat, %	3.69	3.68	0.08	0.89	<0.001	0.18
Fat yield, kg/d	0.65	0.61	0.04	0.20	0.07	0.36
Protein, %	3.12	3.35	0.09	0.001	0.06	0.86
Protein yield, kg/d	0.56	0.52	0.02	0.01	0.001	0.84
Lactose, %	4.49	4.38	0.04	0.005	0.02	0.96
Lactose yield, kg/d	0.79	0.69	0.05	<0.001	<0.001	0.56
Casein, %	2.32	2.48	0.06	0.02	0.12	0.63
Casein yield, kg/d	0.41	0.39	0.02	0.12	0.004	0.95
Log ₁₀ SCC ³	2.17	2.35	0.11	0.20	0.34	0.56
MUN, mg/dL	13.61	12.31	0.37	<0.001	0.008	0.30

¹3.5% FCM = [(0.4324 . milk yield) + (16.216 . fat yield)].

²LI_{95%} = 95% canopy light interception and LI_{Max} = Maximum canopy light interception.

³Logarithmically transformed SCC.

In intensively managed tropical pastures concentrations of protein are typically high (Santos et al., 2014), with most of this protein on intermediate or low digestible fraction as previously discussed. According to Gregorini et al. (2016), cows grazing high crude protein content supplied by the base dietary forage have low ENU leading to loss of nutrients in the system. In the present experiment, ENU (milk N/ N intake) was the same for the two treatments (17.4%, *P* = 0.58; Table 5), which means that 82.6% of N ingested was wasted. These values are commonly found in pasture-based dairy production systems (Gregorini et al., 2016). MUN and ENU have been used as references to monitor protein status of the diet, as well as its energy to protein ratio (Hof et al., 1997; Jonker et al., 1998). Danés et al. (2013) reported 18.4% of ENU for mid lactation cows grazing elephant grass receiving supplementation only with corn grain and vitamin-mineral mix. The ENU decreased to 16.2% when the CP content of the concentrate was increased with soybean meal with no increase in milk and milk protein yields. The low N utilization can also be observed through high excretion of MUN (Table 4), which was above the considered ideal 8.5 to 11.5 mg/dL for lactating cows (Kohn et al.; 2002). Cows on the LI_{95%}, treatment had higher CP intake (3.3 vs 2.7 kg/d, *P* < 0.001) and higher MUN (13.6 vs 12.3 mg/dL, *P* < 0.001). According to Danés et al. (2013) for mid lactation dairy cows producing 20 L/d and grazing intensively managed elephant grass, a diet CP content around 15.5% of DM was sufficient to meet the protein requirements. As for the MUN, the urinary N was higher for LI_{95%} (202.4 vs 181.4 g/ d, *P* = 0.04; Table 5). Fecal N excretion was not changed by treatments (149.8 g/d, *P* = 0.13; Table 4). Urine is the major route for N excretion (Marini

and Van Amburgh, 2005) while the N in feces is minimally altered by dietary CP (Marini and Van Amburgh, 2005; Zanton and Heinrichs, 2008).

Dairy cows grazing forage at LI_{95%} emitted the same amount of CH₄ as those grazing at LI_{max} (296.95 g/d) (Congio et al., 2018). When rumen pH is between 6.0 to 7.0, CH₄ output is maximum and constant (Lana, 1998; Russel, 1998; Janssen, 2010). For dairy cows grazing on tropical pasture, rumen pH is in this range, even under a high concentrate supplementation level (Danés et al., 2013). However, as reported in this study, forage from pastures managed at LI_{95%} had less NDF with less lignin, resulting in greater NDF digestibility and 6% more pdNDF than LI_{max} (Table 3). The increase in fiber digestibility was the major contributing factor for the higher OM digestibility of LI_{95%} and so, to the higher concentration of total volatile fatty acids (67.1 vs 56.9, $P = 0.03$; Table 5), with higher proportion of propionate and less acetate. Despite of not enough to inhibit methanogens, this alteration on the route of fermentation, stimulating propionate production, resulted in lower emission of CH₄ per unit of feed fermented as reported by Janssen (2010). In the present study, enteric CH₄ per net energy intake (g/Mcal) was 18% lower for LI_{95%} (11.6 vs 14.2 g/Mcal, $P = 0.01$).

Table 5. Ruminal parameters and nitrogen using of grazing dairy cows grazing elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) submitted to rotary growth strategies

Item	Treatment (Trt) ¹		SEM	P-value		
	LI _{95%}	LI _{max}		Trt	Time	Trt*time
VFA, mol/100 mol						
Acetate	68.2	68.9	0.17	0.004	<0.001	0.28
Propionate	16.8	16.4	0.15	0.10	<0.001	0.97
Butyrate	11.4	10.9	0.13	0.006	0.06	0.95
Valerate	1.02	0.98	0.017	0.03	0.001	<0.001
Isobutyrate	1.06	1.11	0.029	0.21	<0.001	0.10
Isovalerate	1.54	1.56	0.046	0.80	<0.001	0.04
Acetate: propionate	4.1	4.2	0.04	0.04	<0.001	0.88
Total, mM	67.1	56.9	3.1	0.03	0.01	0.08
Microbial synthesis, g/d	1312.3	1217.8	80.41	0.36	0.004	0.96
CH ₄ /NEL intake, g/Mcal	11.6	14.2	0.74	0.01	0.03	0.02
Urine N, g/d	202.4	181.4	7.69	0.04	0.002	0.69
Fecal N, g/d	154.9	144.7	4.90	0.13	0.98	0.65
ENU ² , %	17.6	17.2	0.89	0.58	<0.001	0.87

¹LI_{95%} = 95% canopy light interception and LI_{max} = Maximum canopy light interception.

²ENU: Efficiency of N utilization calculated for each cow in the lactation trial by dividing mean milk N output (milk CP/6.38) by total N intake.

Table 6. Effects of strategies of rotational grazing management on energy balance

Item (kg)	Treatment (Trt) ⁸		SEM	P-value		
	LI _{95%}	LI _{max}		Trt	Time	Trt*time
NE _L intake ¹ , Mcal/d	25.7	20.8	0.91	<0.001	0.33	0.01
NE _L available maint ² , Mcal/d	9.2	5.1	1.5	0.02	0.69	0.06
NE _L BW gain ³ , Mcal/d	3.3	3.6	1.5	0.80	0.004	0.63
NE _L activity ⁴ , Mcal/d	0.9	0.9	0.03	0.22	0.54	0.93
Milk NE _L ⁵ , Mcal/d	12.3	11.2	0.59	0.03	0.06	0.92
Milk NE _L , Mcal/kg	0.68	0.71	0.01	0.24	0.13	0.69
Efficiency ⁶ , %	47.4	53.8	2.8	0.006	0.75	0.06
BW, Kg	498.7	486.4	14.96	0.29	0.73	0.86
BW change ⁷ , kg	0.44	0.55	0.36	0.61	0.003	0.61
BCS	2.8	2.9	0.03	0.05	0.01	0.12
BCS change ⁷	-0.06	0.01	0.05	0.13	0.83	0.71

¹NE_L(intake) = DMI . NE_L(diet).

²NE_L available for maintenance = NE_L(intake) – NE_L(BW gain) – NE_L(milk) – NE_L(activity).

³NE_L BW gain = BW change . 5.65

⁴NE_L activity = (distance between the milking center and pasture . 0.00045 . BW) + (0.0012 . BW)

⁵NE_L(milk) = Milk yield (kg) . (0.0929 . fat % + 0.0563 . true protein % + 0.0395 . lactose %) (NRC, 2001).

⁶Efficiency: Milk NE_L / NE_L intake.

⁷Calculated as the difference in BW or BCS every period.

⁸LI_{95%} = 95% canopy light interception and LI_{max} = Maximum canopy light interception.

2.4 Conclusions

In intensive pasture-based milk production systems, the management based on LI_{95%} allows lactating cows to have access to pastures with lower proportion of stems, with higher proportions of young leaves with better chemical composition and to perform more efficient grazing with lower forage losses. Therefore, the LI_{95%} pasture management strategy results in higher energy intake and higher milk production per cow, higher stocking rates of pasture, higher milk yield per area, with lower methane emissions per net energy intake compared to management based on LI_{max}. However, dietary N use efficiency does not increase with this management practice.

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3 EFFECTS OF Paddock ALLOCATION TIME ON THE PERFORMANCE, RUMINAL VARIABLES AND NITROGEN UTILIZATION OF MID-LACTATION DAIRY COWS GRAZING ROTATIONALLY MANAGED ELEPHANT GRASS

Abstract

The objective of the present study was to evaluate the effects of paddock allocation time on milk production, ruminal variables and efficiency of N use (ENU) of mid-lactation dairy cows grazing rotationally managed elephant grass. Twenty mid-lactation, multiparous, crossbred (Holstein × Jersey) dairy cows were blocked and assigned randomly to 2 treatments based on time of new paddock allocation for grazing. Animals were allocated to paddocks after the morning (a.m.) or afternoon (p.m.) milking (0630 h and 1630 h, respectively). The pre-grazing and post-grazing management targets corresponded to 95% canopy light interception and 50% of the pre-grazing height, respectively. Cows were fed 1 kg of concentrate (as fed) for every 3 kg.d⁻¹ of milk before each milking. Cows from p.m. treatment grazed forage containing higher concentrations of dry matter (DM) and NSC (nonstructural carbohydrates soluble carbohydrates + starch) and less neutral detergent fiber (NDF) and acid detergent fiber (ADF) compared with cows from a.m. treatment, allowing a higher C:N ratio. The crude protein (CP) and NSC digestibility were higher for the a.m. than for the p.m. treatment. Daily dry matter intake (DMI) and milk yield were not affected by treatments, however, cows from the p.m. treatment presented greater microbial synthesis, less MUN and a tendency for greater milk protein and casein yields. No differences were found on rumen NH₃-N and ENU was higher for the p.m. treatment during the first measurement period. Ruminal molar proportions of branched-chain and butyrate volatile fatty acids (VFA) were lower for cows from the p.m. treatment. Overall, allocating mid-lactation dairy cows to new paddocks of elephant grass in the afternoon, allows them to harvest higher quality forage, to produce milk with less MUN and to synthesize greater amount of microbial protein with a trend for greater production of milk crude protein and casein relative to allocation in the morning.

Keywords: Grazing dairy cow; Nonstructural carbohydrates; Nitrogen use; Tropical grass

3.1 Introduction

The higher content of cell wall on tropical grasses is associated with anatomical aspects of these species due to the high content of vascular tissue, characteristic of C₄ plants compared with C₃ plants (Van Soest, 1994). However, fine tuning pasture management practices allow farmers to produce tropical forage lower in NDF content and with higher fiber digestibility (Palhano et al., 2007; Lopes, 2011; Congio et al. 2018). Intensive grazing systems can provide forage high in CP (De Souza et al., 2017; Congio et al., 2018) but still low in total NSC (Brito et al., 2016) and with an NDF content great enough to cause rumen fill (Allen et al., 2000). All together, these factors limit the supply of total energy required for efficient use of its nitrogen compounds efficiently (Poppi and McLeannan, 1995). According to Kolver (2003), the supply of metabolizable energy was the first-limiting factor for milk production from high-quality

pasture. A synchronism between rumen degradation of protein and dietary carbohydrates is required for optimal microbial growth and protein synthesis (Russell and Hespel, 1981).

The accumulation of carbohydrates by the photosynthetic process continues throughout the day increasing the concentration of sugars in plant tissues, reaching a plateau. In the absence of light, the process is downregulated decreasing the concentration of carbohydrates and sugar (Curtis, 1944; Lechtenberg et al. 1971) in plant tissues. By the effect of light presence, forage grazed at sunset may alter nutrient supply when compared to forage grazed at sunrise (Oliveira et al., 2014; Vibart et al., 2017), increasing DMI (Brito et al., 2008; 2016), digestibility of organic matter (Brito et al., 2008, 2014) and milk yield (Brito et al., 2008). Moreover, forages with higher total nonstructural carbohydrates can alter the ruminal fermentation and optimize the use of nitrogen (Brito et al., 2008).

In environments with high luminous incidence, C₄ plants have higher photosynthesis activity with less photorespiratory activity than C₃ plants, allowing high potential for accumulation of carbohydrates. Most comparisons of carbohydrate accumulation at a.m. or p.m. were made with C₃ plants (Gregorini et al., 2006; Brito et al., 2008; 2014; Vibart et al., 2017) and the effects on metabolism and performance of dairy cows grazing tropical forage is scarce in the literature. In a study with elephant grass (*Pennisetum purpureum* cv Napier), the forage harvested at sunset had higher values of DM and soluble carbohydrate (SC), with less values of CP (Oliveira et al., 2014). The association of techniques of grazing management as presented by Congio et al. (2018), associated with the time of paddock allocation, can make systems based on pastures more efficient and productive.

We hypothesized that, in a rotationally grazed elephant grass pasture, new paddock allocation in the afternoon compared with allocation in the morning, would improve N utilization, DMI, and milk yield of mid-lactation dairy cows. The objective of this study was to evaluate the effects of new paddock allocation time on the performance, ruminal variables and nitrogen utilization of dairy cows grazing rotationally managed elephant grass.

3.2 Materials and methods

The study was conducted at ESALQ-USP in Piracicaba, Sao Paulo, Brazil, on an elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) pasture established in the early 1970's and managed under rotational grazing ever since. All procedures involving animals were approved by the Ethical Committee for Animal Research of the University of Sao Paulo, Piracicaba (protocol: 2016-20).

3.2.1 Treatments and grazing Management

Treatments were allocated to experimental units (2064 m² paddocks) according to a randomized complete block design, with eight replications. The slope and chemical soil characteristics were considered as blocking criteria. Each paddock was divided up into three sub-paddocks (688m²) with forage measurements performed within the central sub-paddock. The treatments corresponded to the time of the day dairy cows were allocated to a new paddock: in the morning (a.m.; ~ 0630 h, following the morning milking) or in the afternoon (p.m.; ~ 1630 h following the afternoon milking). The cows were kept 1 day in each paddock, which were used according to a rotational grazing management strategy. The pre-grazing target of canopy height was 100 cm, which corresponded to 95% interception of the incident light by the canopy (Congio et al., 2018) and the post-grazing target height was 50% of the pre-grazing height (around 50 cm). The pasture was fertilized with 56 kg of N/ha after each grazing. All grazing-related measurements were taken in a daily basis. Sward height was monitored using a stick graduated in centimeters to take 40 randomized readings from each paddock. Herbage mass and morphological composition were determined at pre-grazing harvesting all forage contained within a 1.25 x 0.75 m metallic frame. Frames were allocated in 2 representative areas of the paddocks at the time of sampling (visual assessment of canopy height and herbage mass) and herbage cut at the targeted post-grazing height. Samples were weighed and a representative subsample was taken and separated into leaf blades, stems (including leaf sheaths), and dead material to determine the morphological composition. Morphological composition is presented in Table 1. Samples of the grazed stratum were taken along with other measurements before animals entered the paddock. Pasture samples were lyophilized (Liotop, São Carlos, SP, BRA) at -50 °C, ground through a 1mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA) and stored at room temperature until chemical analysis.

3.2.2 Lactation and Ruminant Metabolism Trial

Twenty multiparous crossbred (Holstein × Jersey) cows were used, averaging (mean ± SD) 102 ± 82 DIM, 18 ± 4 kg of milk/d and 461 ± 72 kg of BW at the beginning of the trial. All cows received the same amount of concentrate feed for a standardization period of 21 d, after which they were blocked for similar milk yield, DIM and BW, with milk yield being the higher order criterion. The experiment was carried out from January to March 2017 (56 days),

comprising 2 sampling periods (28 days each one), with samplings performed during the final ten days. Concentrate feed was fed individually twice a day at 4:30 a.m. and 3:00 p.m., before each milking, at a rate of 1 kg of concentrate (as fed) for every 3 kg of milk, using as reference the milk yield recorded at the end of the standardization period for each block and revised at the end of each sampling period. The concentrate feed was composed of fine ground corn (80%), soybean meal (15%) and a mix of minerals and vitamins (5%), resulting in 13.6% CP. Samples of concentrate feed were collected during 5 consecutive days at the end of each sampling period, composited by period and stored at room temperature for chemical analysis. Milk yield was measured on a daily basis and milk composition during the last ten consecutive days of each sampling period. Milk samples from both milking were stored in vials with a bronopol (Advanced Instruments, Norwood, MA) preservative pill and sent immediately for composition analysis. Forage intake and nutrient digestibility were measured at the end of each sampling period from total fecal excretion and feed indigestibility. To determine fecal excretion, TiO_2 was dosed twice a day (20 g/cow per day) after each milking for 12 d. Fecal grab samples were collected after the morning and afternoon milking during the last 5 d of each sampling period, and stored in a -20°C freezer. Subsequently, samples were thawed at room temperature, composited by cow, dried at 55°C in a forced-air oven, and ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA). To calculate *in vivo* feed digestibility (concentrate and grazed stratum samples), the internal marker indigestible neutral detergent fiber (iNDF) was estimated using an *in vitro* incubation in buffered rumen fluid for 240 h by the *in vitro* procedure described by Goeser and Combs (2009). In order to estimate microbial synthesis, the purine derivative excretion method of Chen and Gomes (1992) was used. Spot samples of urine were collected 4 h after the morning concentrate feeding during the last day of each sampling period (Valadares et al., 1999) Ten milliliters of urine were mixed with 40 mL of 0.072 N of H_2SO_4 and stored at -20°C . Rumen fluid was sampled with a flexible orogastric tube from all cows at the end of each sampling period 4 h after the morning concentrate feeding. Samples were instantaneously frozen in liquid nitrogen to suppress fermentation and kept frozen at -20°C until preparation for analysis.

3.2.3 Chemical Analysis and Calculations

Composite forage, concentrate, and fecal samples were analyzed for DM (drying samples in an oven at 105°C for 24 h), ash (AOAC International, 2005; method 942.05), total N content (Leco FP-2000 N Analyzer; Leco Instruments Inc., St. Joseph, MI), ether extract

(method 920.85; AOAC, 1986), NDF (Van Soest et al., 1991) with sodium sulfite and heat-stable α -amylase for concentrate samples and ADF and lignin (AOAC International, 2005; method 973.18) for feed samples. Herbage samples from the grazed stratum were also analyzed for neutral detergent insoluble protein (NDIP), acid detergent insoluble protein (ADIP) without sodium sulfite (Van Soest et al., 1991), nonprotein nitrogen (NPN) (Licitra et al., 1996), soluble carbohydrate (SC) and starch by Hall (2003). Fractions of protein and carbohydrate were calculated by the CNCPS system (Sniffen et al., 1992). Protein: A+B1 (NPN + rapidly degraded protein, respectively), B2 (protein with intermediate rates of degradation), B3 (slowly degraded protein) and C (unavailable N). Carbohydrate: total carbohydrate (TC) = $100 - (\%CP + \%EE + \%ash)$; A (sugar); B1 (starch and nonstructural polysaccharides); B2 (available fiber) and C (unavailable fiber). Forage intake was calculated from total fecal excretion and feed indigestibility. To determine fecal excretion, fecal samples were analyzed for titanium concentration according to Myers et al. (2004). Feed and fecal samples were analyzed for indigestible NDF (NDF remaining after 240 h *in vitro* incubation). Total fecal excretion, fecal excretion coming from the concentrate and forage intake were calculated according to De Souza et al. (2015). Urine samples were thawed and analyzed for creatinine, allantoin, and uric acid using HPLC. Total urine production was estimated by creatinine content, assuming that the excretion of creatinine was constant at 0.213 mmol/kg BW (Chizzotti et al. 2008). Apparent ENU (assuming no retention or mobilization of body N) was calculated for each cow by dividing mean milk N output (milk CP/ 6.38) by total N intake. Urinary N excretion was calculated from total urine production and its N concentration. Milk samples were analyzed for fat, protein, lactose, total solids (TS), and MUN using infrared procedures (Foss 4000; Foss North America, Eden Prairie, MN). Ruminant fluid samples were thawed at room temperature, centrifuged ($15,000 \times g$, 4°C, 30 min), and the supernatant was analyzed for VFA by gas chromatography (Palmquist and Conrad, 1971).

3.2.4 Statistical Analysis

The data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). Within forage parameters, paddocks were considered the experimental unit, and for animal measurements, the cow was considered the experimental unit. All variables were analyzed as repeated measures. The statistical model used was:

$$Y_{ijk} = \mu + B_i + C_j + T_k + CT_{jk} + e_{ijk}$$

Where Y_{ijkl} is the variable of interest, μ is the overall mean, B_i is the random effect of block ($i = 1$ to 10 for animals and 1 to 8 for forage), C_j is the fixed effect of treatment ($j =$ a.m. and p.m.), T_k is the fixed effect of period ($k =$ period), CT_{jk} is the fixed effect of interaction between treatment and period, and e_{ijk} is the residual error. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Data were considered outliers and excluded from the data set when a Student's residual higher than 2 or lower than -2 was detected. Denominator degrees of freedom were adjusted by the Kenward-Rogers method. Significance was determined at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

3.3 Results and discussion

Management using the 95% canopy light interception criterion ($LI_{95\%}$) as the pre-grazing target was used since it has been demonstrated it improves sward structure and forage nutritive value (Da Silva et al., 2017; Congio et al. 2018). The pre- and post-grazing canopy heights for the a.m. and p.m. treatments were 101.6 and 55.7, and 100.4 and 54.2 respectively. Grazing period and stocking rate were the same for both treatments (1 d and 6.1 cows/ha, respectively). Time of new paddock allocation to dairy cows did not alter structural characteristics of the canopy (Table 1). Forage allowance was the same for both treatments, 15.2 kg DM per cow/d (estimated from herbage above the targeted post-grazing height). Congio et al. (2018), in the same experimental area, evaluated the effects of strategies of rotational grazing management (95% or maximum LI during regrowth as pre-grazing targets) on canopy structure and morphological composition of elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) and found higher leaf:stem ratio, lower grazing losses and shorter defoliation intervals allowing more grazing cycles (5.6 vs 3.5) and an increase in the stocking rate (9.3 vs 7.0 cows/ha) for the $LI_{95\%}$ relative to the LI_{Max} treatment. In the present study, herbage mass was comprised of 98.8% leaves, with no dead material above the post-grazing height.

Table 1. Pre-grazing structural characteristics of rotationally grazed elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) with new paddocks allocated to dairy cows in the morning (a.m.) or in the afternoon (p.m.)

Characteristic ¹	Treatment (Trt)		SEM	P-value		
	a.m.	p.m.		Trt	Period	Trt*Period
	Herbage mass, kg of DM/ha	2270	2180	208.5	0.67	0.68
<u>Morphological composition, % of Herbage Mass</u>						
Leaves	98.2	99.3	0.55	0.08	0.04	0.01
Stem	1.8	0.7	0.55	0.08	0.04	0.01
Dead material	-	-	-	-	-	-
Density, kg of DM/cm	57.9	50.0	4.39	0.24	0.5	0.74

¹All measurements were taken above the post-grazing residue (50% of the pre-grazing height).

Forage composition differed among treatments (Table 2); p.m. forage had greater DM (22.3 vs 18.9%; $P < 0.001$), SC (8.2 vs 5.4% of DM basis; $P < 0.001$) and Starch (2.9 vs 1.5% of DM basis; $P < 0.001$) concentrations and smaller NDF (60.0 vs 61.8% of DM basis; $P = 0.013$) and ADF (34.4 vs 36.8% of DM basis; $P = 0.005$) concentrations than a.m. forage. These results are in agreement with studies using *temperate* (Brito et al., 2008, 2017; Vibart et al., 2017) and *tropical grasses* (Oliveira et al., 2014). In studies comparing a.m. and p.m. harvested forage, there was a 85.4 and 22.8% increase in SC for tropical grass (Oliveira et al., 2014) and temperate grass (Delagarde et al., 2000), respectively, in the p.m. relative to the a.m. forage. Net photosynthesis and evapotranspiration losses increase forage non-structural carbohydrate and DM concentrations (Gregorini et al., 2006), often diluting fiber and/or N concentrations in forage DM (Delagarde et al., 2000).

Table 2. Herbage chemical composition of rotationally grazed elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) with new paddocks allocated to dairy cows in the morning (a.m.) or in the afternoon (p.m.)

Item, % of DM	Treatment (Trt)		SEM	P-value		
	a.m.	p.m.		Trt	Period	Trt*Period
DM	18.9	22.3	0.67	<0.001	<0.001	0.72
Organic matter	90.5	90.6	0.42	0.79	<0.001	0.46
Ash	9.6	9.4	0.418	0.79	<0.001	0.46
CP	17.6	17.1	0.42	0.33	0.25	0.88
NDF	61.8	60.0	0.56	0.013	<0.001	0.18
ADF	36.8	34.4	0.54	0.005	0.93	0.35
Lignin	3.4	3.4	0.16	0.895	0.12	0.94
Ether extract	3.1	3.0	0.08	0.66	0.022	0.008
Soluble carbohydrates	5.4	8.2	0.27	<0.001	0.90	0.79
Starch	1.5	2.9	0.13	<0.001	0.16	0.59
NSC/CP ¹	0.52	0.87	0.040	<0.001	.	.

¹NSC (soluble carbohydrates + starch)/ CP (fractions: A + B1 + B2; Sniffen et al., 1992).

Carbohydrate and protein fractions in the forage are presented in Table 3. There was no difference between a.m. and p.m. treatments for forage CP, soluble protein (A+B1 fractions) and medium degraded protein (B2 fraction) ($P > 0.05$). The CP levels of forage plants and their soluble fractions (A+B1) are highly influenced by the N rates applied after each cut or grazing (Johnson et al., 2001) which were the same for both treatments in the present study. The CP fraction B3 (percentage of CP in the feedstuff that is slowly degraded protein; Sniffen et al., 1992) was higher in the p.m. forage (17.5 vs 16.0% of CP; $P = 0.010$) than in the a.m. forage. In intensively managed grazing systems the high demands for N can result in high soluble and rapidly degradable nature of pasture protein (Kolver, 2003). This characteristic associated with the low level of non-structural carbohydrate in both high-quality tropical and temperate pastures (Kolver, 2003), lead to a poor N utilization by grazing cattle (Poppi et al., 1995; Gregorini et al., 2016).

Table 3. Protein and carbohydrate fractions (% of CP and % of TC) of rotationally grazed elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) with new paddocks allocated to dairy cows in the morning (a.m.) or in the afternoon (p.m.)

Item ¹	Treatment (Trt)		SEM	P-value		
	a.m.	p.m.		Trt	Period	Trt*Period
CP, % of DM	17.6	17.1	0.42	0.33	0.25	0.88
A+B1	25.9	23.9	1.11	0.21	0.19	0.91
B2	51.9	51.5	1.10	0.77	0.01	0.84
B3	16.0	17.5	0.37	0.01	<0.001	0.34
C	6.6	6.6	0.31	0.79	0.41	0.37
TC, % of DM	69.8	70.5	0.67	0.38	0.004	0.45
A	13.8	15.2	0.49	0.012	0.42	0.25
B1	3.3	5.7	0.26	<0.001	0.10	0.48
B2	71.2	67.6	0.66	<0.001	0.15	0.16
C	11.7	11.5	0.54	0.63	0.09	0.80

¹ Fractions of protein and carbohydrate were calculated by the CNCPS system (Sniffen et al., 1992). Protein: A+B1 (non-protein nitrogen + rapidly degraded protein, respectively), B2 (protein with intermediate rates of degradation), B3 (slowly degraded protein) and C (unavailable N). Carbohydrate: total carbohydrate (TC) = 100 – (%CP + %EE + %ash); A (sugar); B1 (starch and nonstructural polysaccharides); B2 (available fiber) and C (unavailable fiber).

Besides the analyses of forage for NDF, ADF, SC and starch concentration presented in Table 2, the carbohydrate fractions of forage from both treatments were also analyzed according to Sniffen et al. (1992) (Table 3). Sugar, starch and nonstructural polysaccharides (A + B1 fractions) contents, rarely exceed 20% of TC in tropical grasses (Vieira et al., 2000). The concentrations of carbohydrate fraction A (15.2 vs 13.8% of TC; $P = 0.0115$) and B1 (5.7 vs 3.3%; $P < 0.001$) were higher and the concentrations of fraction B2 (67.6 vs 71.2%; $P < 0.001$) was smaller in the p.m. forage compared with the a.m. forage, corroborating the data from Table 2.

A significant treatment \times period interaction was observed on intake of DM, OM, CP and NDF, that fluctuated between the periods and did not result in a significant effect of treatments (Table 4). Cows on both treatments had access to paddocks for the same number of hours per day and were offered the same daily forage allowance. Our study is in agreement with others that compared a.m. versus p.m. paddock allocation time. Vibart et al. (2017) used late-lactation dairy cows grazing ryegrass (*Lolium perenne* L.) and Gregorini et al. (2006) used beef heifers grazing ryegrass (*Lolium multiflorum* Lam.) and both also did not find differences in DMI. However, an increase of 0.7 kg/d on DMI was observed by Brito et al. (2016) for mid-lactation dairy cows fed with a total mixed ration formulated with p.m.-cut timothy baleage. Studies with beef heifers showed that animals preferentially select forages with high

concentrations of NSC, leading to an increase in DMI (Huntington and Burns, 2007; Mayland et al., 2000). In the present study, the higher SC ($P < 0.001$) and starch ($P < 0.001$) concentrations in the p.m. forage (Table 2) did not stimulate cows to consume more forage and their 6% higher intake of NSC ($P = 0.02$) was offset by the lower NSC (-3.4%, $P = 0.01$) and CP (-3.9%, $P = 0.01$) digestibility (Table 4). The content of NDF in forages affect its capacity of causing rumen fill what may limit cow intake (Allen, 2000). Apparently, the lower NDF content of the p.m. forage (60.0 vs 61.8%; $P = 0.013$) was not enough to decrease rumen fill and stimulates cow forage intake. Digestibility of NDF is another potential factor causing rumen fill (Allen, 2000). The absence of difference ($P > 0.05$) in NDF digestibility (56.96%) between p.m. and a.m. treatments was expected once lignin content (3.4%) of both forages was similar ($P > 0.05$).

Table 4. Nutrients intake and apparent total tract diet digestibility of dairy cows on rotationally grazed elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) with new paddocks allocated to dairy cows in the morning (a.m.) or in the afternoon (p.m.)

Item	Treatment (Trt) ²		SEM	P-value		
	a.m.	p.m.		Trt	Period	Trt*Period
<u>Intake, kg/d</u>						
Total DMI	17.3	17.3	0.45	0.96	0.53	0.003
Forage DMI	11.6	11.6	0.39	0.97	0.27	0.002
OM	16.48	16.54	0.42	0.92	0.38	<0.001
CP	2.94	2.88	0.08	0.54	0.47	<0.001
NDF	8.03	7.87	0.24	0.63	0.06	<0.001
Ether extract	0.61	0.61	0.02	0.84	0.21	0.24
NSC ¹	4.89	5.18	0.13	0.02	0.28	0.25
<u>Digestibility, %</u>						
DM	62.87	62.30	0.66	0.39	0.11	0.50
Organic matter	67.89	67.29	0.64	0.34	0.00	0.16
CP	66.53	63.95	0.80	0.01	0.01	0.52
NDF	56.97	56.94	0.81	0.98	0.00	0.15
Ether extract	73.80	73.51	0.96	0.78	0.16	0.53
NSC ¹	86.41	83.45	0.85	0.01	<0.001	0.03

¹Nonscrutural carbohydrates: (soluble carbohydrates + starch)

Time of paddock allocation did not affect ($P > 0.05$) milk yield (17.3 kg/d), 3.5% FCM yield (17.05 kg/d), milk fat content (3.5%) and yield (0.595 kg/d), protein content (3.25%), casein content (2.55%), lactose yield (0.78 kg/d), and total solids content (12.25%) and yield (2.1 kg/d). However, the p.m. treatment resulted in lower ($P < 0.01$) milk lactose content (4.6 vs 4.4%) and MUN (14.6 vs 13.0 mg/dl) and a tendency ($P < 0.10$) to increase yields of milk protein (0.58 vs 0.55 kg/d) and casein (0.45 vs 0.42 kg/d) (Table 5). The higher concentration

of NSC in the forage from paddocks allocated to cows in the afternoon can improve the fermentable carbon to nitrogen (C:N) ratio (Table 2), and so improve the incorporation of ammonia into microbial protein (Table 6). This would result in more milk protein synthesis and secretion (Higgs et al., 2013) as well as in enhanced N utilization efficiency (Brito et al., 2008). Batistel et al. (2017) reported increased milk protein and casein contents and yields when a more degradable starch source as steam-flaked flint corn replaced fine ground corn for grazing cows. On the other hand, increasing crude protein content of the concentrate (8.7 vs 13.4 vs 18.1% CP) fed to cows grazing tropical grass (18.5, 9.2% of CP and NSC on DM basis, respectively) had no effect on milk protein and casein content and yields (Danés et al., 2013). Brito et al. (2008), using late-lactation dairy cows fed with a total mixed ration based on p.m.-cut timothy baleage and no concentrate, showed an increase in DMI (+5%), ECM (+8%), milk N efficiency (+8%) and bacterial protein synthesis (+7%), suggesting that the effect of increased NSC in the forage was more effective on the performance without grain supplementation. Another point to consider is grazing behavior, the major grazing event on ruminants occurs during the evening and is the longest and most significant in terms of forage intake (Gregorini et al., 2012). Hormonal (i.g. melatonin and serotonin; Gregorini et al., 2012) and ambient temperature can influence grazing behavior. In the present study, average maximum daily temperature was 31.03 °C. Elevated temperatures during the study, may have led cows to select cooler periods for grazing, such as early in the morning or late in the afternoon. Cows allocated to new paddocks in the morning have more grazing events throughout the day than cows allocated to new paddocks in the afternoon, however even the a.m. cows spent more time grazing at night (Gregorini et al., 2012). The pattern grazing behavior at p.m. may have diluted the effect of the treatments.

Table 5. Milk yield and composition of dairy cows on rotationally grazed elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) with new paddocks allocated to dairy cows in the morning (a.m.) or in the afternoon (p.m.)

Item	Treatment (Trt)		SEM	P-value		
	a.m.	p.m.		Trt	Period	Trt*Period
Milk, kg/ d	17.2	17.4	1.20	0.66	0.10	0.88
3.5% FCM ¹	17.1	17.0	1.10	0.89	0.23	0.46
Fat, %	3.5	3.5	0.13	0.63	0.94	0.22
Fat yield, kg/ d	0.59	0.60	0.03	0.92	0.26	0.56
Protein, %	3.2	3.3	0.10	0.30	0.04	0.12
Protein yield, kg/ d	0.55	0.58	0.02	0.09	0.18	0.37
Lactose, %	4.6	4.4	0.06	<0.001	0.11	0.16
Lactose yield, kg/ d	0.79	0.77	0.05	0.54	0.05	0.72
Casein, %	2.5	2.6	0.09	0.23	0.19	0.03
Casein yield, kg/ d	0.42	0.45	0.02	0.06	0.10	0.10
Total solids, %	12.3	12.2	0.25	0.68	0.37	0.81
Total solids, kg/ d	2.1	2.1	0.11	0.92	0.04	0.82
Log ₁₀ SCC ²	2.06	2.35	0.12	0.08	0.78	0.91
MUN, mg/dL	14.6	13.0	0.46	0.003	0.054	0.22

¹3.5% FCM = [(0.4324. milk yield) + (16.216. fat yield)].

²Logarithmically transformed SCC

The N use is presented on Table 6. A treatment × period interaction was observed for ENU and N intake ($P < 0.05$). The higher ENU (20.9 vs. 17.58 %, $P = 0.03$) for the p.m. treatment was associated with lower N intake (481.4 vs. 502 g/ d; $P = 0.009$) on first period, with no difference in the second. In a simulation modeling exercise, Gregorini et al. (2010) showed that allocating pasture during the afternoon can reduce urine N discharges on the pasture, reducing environmental impact. Brito et al. (2014), feeding early-lactation cows alfalfa baleage with different concentrations of NSC and supplementing with a common corn-based concentrate, did not find improvement on milk yield and N utilization.

The greater supply of highly fermentable carbohydrate resulted in more microbial protein (+ 63.3 %, $P = 0.029$) and less MUN (-10.95 %, $P = 0.0032$) for cows allocated to new paddocks in the afternoon than in the morning (Table 6), however, no difference on NH₃-N concentration (11.05 mg/dL) was observed between treatments (Table 7). Greater microbial yield can result in greater supply of higher quality metabolizable protein for dairy cows (NRC, 2001) and support the trend for higher yields of milk protein and casein of cows allocated to new paddocks in the afternoon. Greater microbial yield has been reported consistently when more fermentable carbohydrates are fed to dairy cows (NRC, 2001), however the differences in concentration of fermentable carbohydrate between a.m and p.m. forage cannot explain the

large difference in microbial yield between treatments in the present study. Therefore, factors such as unrepresentative urinary sampling and analytical errors should not be ruled out.

Table 6. Nitrogen utilization efficiency of dairy cows on rotationally grazed elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) with new paddocks allocated to dairy cows in the morning (a.m.) or in the afternoon (p.m.)

Item	Treatment (Trt)		SEM	P-value		
	a.m.	p.m.		Trt	Period	Trt*Period
ENU ¹ , %	18.60	19.62	0.86	0.30	0.80	0.02
Microbial synthesis ² , g/ d	708.57	1118.62	145.53	0.05	0.98	0.70
N intake, g/ d	470.92	461.36	12.21	0.54	0.47	0.002
N Urine, g/ d	142.62	179.45	20.06	0.20	0.90	0.37
N Fecal, g/ d	120.87	124.89	3.54	0.43	0.24	0.12

¹ ENU: Efficiency of N utilization calculated for each cow in the lactation trial by dividing mean milk N output (milk CP/6.38) by total N intake.

² Estimated microbial synthesis was derived from urine volume production by the creatinine content of each sample, assuming that the excretion of creatinine was constant at 0.213 mmol/kg BW (Chizzotti et al., 2008) and purine derivative excretion method of Chen and Gomes (1992) using 0.116 as purine: N constant.

Rumen fermentation parameters are presented on Table 7. Paddock allocation time had no effect ($P > 0.05$) on rumen NH₃-N, molar proportions of acetate and propionate and acetate:propionate ratio. Cows from the p.m. treatment presented lower rumen molar proportion of butyrate ($P = 0.007$) and a treatment \times period interaction ($P < 0.05$) with lower rumen molar proportions of isobutyrate (1.5 vs. 1.6 mM/100mM; $P < 0.001$) and isovalerate (2.2 vs. 1.72 mM/100mM; $P < 0.001$) during the second sampling period. Ruminal branched-chain VFA (isobutyrate, isovalerate and 2-methylbutyrate) are derived from feed protein degradation (Eugène et al., 2004) and microbial protein recycling (Miura et al., 1980) and are essential nutrients for growth of ruminal cellulolytic bacteria (NRC, 2001). The lower rumen molar proportions of isobutyrate and isovalerate may be explained by the greater microbial yield of the p.m. treatment. Brito et al. (2014; 2016) also found less branched-chain VFA (valerate) and no significant difference in propionate concentrations in cows feeding high-NSC.

Table 7. Ruminal parameters of dairy cows on rotationally grazed elephant grass (*Pennisetum purpureum* Schum. cv Cameroon) with new paddocks allocated to dairy cows in the morning (a.m.) or in the afternoon (p.m.)

Item	Treatment (Trt)		SEM	P-value		
	a.m.	p.m.		Trt	Period	Trt*Period
Ammonia-N, mg/dL	10.5	11.6	0.64	0.25	0.25	0.24
VFA, mM/100mM						
Acetate	58.2	58.03	0.58	0.80	0.31	0.85
Propionate	24.4	25.1	0.47	0.18	0.16	0.29
Butyrate	13.1	12.5	0.22	0.007	<0.001	0.66
Valerate	1.08	1.03	0.04	0.20	0.48	0.08
Isobutyrate	1.5	1.3	0.06	0.005	0.035	<0.001
Isovalerate	2.2	1.9	0.10	0.016	0.008	0.01
Acetate: propionate	2.4	2.3	0.06	0.20	0.67	0.35

3.4 Conclusions

In intensive pasture-based milk production systems, allocating cows to new paddocks in the afternoon has no effect on forage intake and milk production of mid-lactation dairy cows. However, the higher NSC content of the forage from the p.m. pastures increases the yield of microbial protein, decreases MUN and tends to increase the yields of milk protein and milk casein compared to a.m. pastures.

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4 CONCLUSIONS

Grazing management based on $LI_{95\%}$ as a pre-grazing target is better than LI_{Max} to improve performance of dairy cows and reducing MUN. Allocating cows in a new paddock on p.m. along with $LI_{95\%}$ improve forage chemical composition and tend to improve performance of mid-lactation cows.