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Strategic grazing management decreases nitrogen excretion intensity of dairy cows

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ABSTRACT: There is limited information regarding both nitrogen (N) and energy partitioning of dairy cows grazing well–managed tropical pastures. The objective of this study was to investigate the N and energy partitioning of mid–lactation dairy cows on rotationally grazed elephant grass using two pre–grazing targets: 95 % or maximum canopy light interception ($LI_{95\%}$ or LI_{Max}) during regrowth. The study used 26 Holstein × Jersey dairy cows arranged in a randomized complete block design with three 40–day periods of sampling. Grazing at $LI_{95\%}$ increased organic matter and crude protein intake by 20 % ($p \leq 0.05$) which resulted in a 9 % increase in fat corrected milk yield ($p \leq 0.05$) relative to LI_{Max}. Cows grazing at $LI_{95\%}$ had greater concentration of total volatile fatty acids, butyrate and valerate ($p \leq 0.05$), and smaller acetate ($p \leq 0.05$) than those grazing at LI_{Max}. Intake of net energy for lactation (NE_L) and NE_L secreted in milk were greater ($p \leq 0.05$), while partitioning of NE_L towards maintenance tended to be greater ($p = 0.07$) for cows grazing at $LI_{95\%}$ than those grazing at LI_{Max}. Milk urea nitrogen and both urine and fecal N excretion were greater for cows grazing at $LI_{95\%}$ ($p \leq 0.05$), but N excretion intensity was lower than in cows grazing at LI_{Max} ($p \leq 0.05$). Strategic grazing management using the $LI_{95\%}$ pre–grazing target increases N losses through both urine and feces; however, it reduces N excretion intensity of dairy cows by 9 %.

Keywords: canopy light interception, elephant grass, energy partitioning, nitrogen partitioning, pasture–based systems

Introduction

Most dairy operations in the tropics are represented by pasture–based systems (Congio et al., 2019). Therefore, the adoption of adequate grazing management strategies become essential to optimize the efficiency of those systems (Congio et al., 2018). The procedure of rotating grazed pastures based on critical leaf area index (LAI) and canopy light interception (LI) has shown effectiveness to increase herbage production and nutritive value (Da Silva et al., 2015). Several studies showed that regrowth interruption when canopy LI reaches 95 % minimizes stem elongation and senescence, generating leafy swards with greater herbage nutritive value, favoring animal performance (Voltolini et al., 2010) and contributing to the reduction of GHG gases emissions (Congio et al., 2018, 2019).

However, these intensively managed systems are usually coupled with moderate or high doses of nitrogen (N) fertilizers resulting in high herbage crude protein (CP) content at the expense of energy content (Danes et al., 2013), which could lead to CP overfeeding of grazing dairy cows (NRC, 2001). When it is not retained in the animal tissues or secreted into milk, N is excreted by urine and feces, causing environmental issues, such as water pollution and gaseous N emissions (Hristov et al., 2019). Studies have reported benefits of mid–lactating dairy cows grazing at $LI_{95\%}$, but there is scarce information detailing pathways and metabolism (Voltolini et al., 2010; Congio et al., 2018). Therefore, understanding both N and energy partitioning and identifying feed management strategies that may improve N–use efficiency (NUE) for productive purposes should be regarded as top priority in ruminant nutrition research (Hristov et al., 2019; Silva et al., 2019). The objective of this study was to investigate N and energy partitioning in mid–lactation dairy cows on rotationally grazed elephant grass affected by pre–grazing targets of either 95 % or maximum canopy LI during regrowth ($LI_{95\%}$ and LI_{Max}, respectively). We hypothesized that CP intake increases in dairy cows grazing at $LI_{95\%}$, leading to greater N excretion through urine and feces; however, it could be compensated by greater milk yield, allowing the reduction of N excretion intensity.

Materials and Methods

This study was conducted according to research protocols approved by the Animal and Environment Ethic Committees at the University of São Paulo, Luiz de Queiroz College of Agriculture (USP/ESALQ) (protocol number 17.5.999.11.9).

Study site and treatments

The experiment was carried out in the municipality of Piracicaba, São Paulo, Brazil (22°42′ S, 47°38′ W, altitude of 546 m a.s.l.) on a rainfed elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon) pasture established in 1972 on a high fertility Eutroferric Red Nitossol. The 2.5 ha experimental area comprised 12 paddocks of 2,058 m² (6 replications per treatment), which were divided into three 688 m² management paddocks each, totaling 18 management paddocks per treatment. Each set of 18–management paddocks formed a self-contained farmlet that was used to feed two groups of dairy cows continuously throughout the experimental period. Grazing management treatments were allocated to experimental units (2,058 m^2 paddocks) in a randomized complete block design. The climate in the region is humid sub-tropical with warm and wet summers and dry winters, and accumulated rainfall averaging 1328 mm during the year (Alvares et al., 2013). The experiment lasted 120 days from Dec 2015 to Apr 2016, during the warm and wet season, after a previous 10-month adaptation period when paddocks were subjected to defoliation regimes (*i.e.,* pre-and post-grazing targets), but no measurements were taken (Congio et al., 2018). The adaptation period and the study of Voltolini et al. (2010), which was carried out in the same experimental site, were used to define the area necessary to support the number of group of cows in this study.

The two treatments corresponded to pre-grazing targets of either 95 % or maximum canopy LI during regrowth ($LI_{95\%}$ and LI_{Max}, respectively). For both treatments, post-grazing heights corresponded to 50 % of the pre-grazing height. We fractioned 215 kg N ha^{-1} throughout the experimental period for both treatments and applied always at post-grazing. Congio et al. (2018) detailed further information on monitoring and control of the experimental conditions.

Herd, feeding and sampling

The experiment comprised 26 Holstein × Jersey dairy cows averaging 488 ± 60 kg (mean ± SD) of body weight (BW), 126 ± 90 days in milk (DIM) and 20.3 ± 2.6 kg d^{-1} of milk at the beginning of the experiment. All cows received the same amount of concentrate for a preliminary period of 15 days and were stratified and grouped into pairs forming 13 blocks according to daily milk yield and DIM. Then, the animals were randomly assigned to farmlets managed at either $LI_{95\%}$ or LI_{Max}. Additional dry-cows were kept in adjacent elephant grass paddocks and were used when necessary to adjust stoking rate and ensure management targets. The concentrate was fed individually twice a day at 4h30 a.m. and 2h30 p.m., before each milking, at a rate of 1 kg of concentrate / 3 kg of milk yield (considering the average of each block). The amount of concentrate was adjusted at the beginning of each sampling period (Congio et al., 2018). Cows were weighed at the beginning of the experiment and at the end of each sampling period during three consecutive days after the morning milking.

Measurements were performed during three periods that totaled 40 days (P_1, P_2 and P_3), the last seven days were used for sampling. Forage intake was estimated from total fecal excretion and feed indigestibility, using titanium dioxide (TiO_2) and indigestible neutral-detergent fiber (iNDF) as markers. To estimate total fecal excretion, TiO_2 was dosed with 10 g cow^{-1} twice a day after concentrate meals for 12 days. Fecal grab samples were collected after morning and afternoon milking on the last five days of each sampling 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-draught oven, and ground through a 1-mm screen. To calculate *in vivo* nutrients digestibility, the iNDF of fecal and feed samples was estimated using an *in vitro* incubation in buffered rumen fluid for 240 h (Goeser and Combs, 2009). Total fecal excretion, fecal excretion coming from the concentrate (FEC), forage intake, dry matter (DM), organic matter (OM), and NDF apparent digestibility were calculated as follows: Total fecal excretion (g d^{-1}) = amount marker dosed daily (g d^{-1}) / concentration marker in feces (g d^{-1}); FEC (kg d^{-1}) = concentrate intake (kg d^{-1}) × concentrate indigestibility (%); Forage intake (kg d^{-1}) = (Total fecal excretion (kg d^{-1}) – FEC (kg d^{-1})) / forage indigestibility (%); DM, OM or NDF digestibility (%) = (DM, OM or NDF intake – DM, OM or NDF total fecal excretion) / DM, OM or NDF intake × 100.

An *in vitro* assay was also conducted to estimate total tract NDF digestibility (TTNDFD), rumen potentially digestible NDF (pdNDF), and pdNFD degradation rate (kd) of herbage samples using a model described by Lopes et al. (2015). The method for purine derivative excretion of Chen and Gomes (1992) was used to estimate microbial synthesis. Spot samples of urine were collected 4 h after feeding the morning concentrate on the last day of each sampling period. Ten milliliters of urine were mixed with 40 mL of 0.072 *N* of H_2SO_4 and stored at –20 °C. Urine samples were thawed and analyzed for creatinine, allantoin, and uric acid using high performance liquid chromatography (Pimpa et al., 2001). The urine N was also analyzed using micro-Kjeldahl procedure (AOAC, 1990) in order to compare N metabolism between treatments. Total urine production was estimated by the creatinine content, assuming that the excretion of creatinine was constant at 0.213 mmol kg BW^{-1} (Chizzotti et al., 2008). Apparent NUE was calculated for each cow by dividing average milk N output (milk CP / 6.38) by total N intake (assuming no retention or mobilization of body N). The intensity of N excretion was calculated by dividing N excreted through both urine and feces by milk yield.

Rumen fluid was sampled through esophageal probe from all cows at the end of each sampling period 4 h after feeding the morning concentrate. A sample of 50 mL of ruminal fluid was filtered and immediately stored at –20 °C to determine the volatile fatty acid (VFA). Ruminal fluid samples were thawed at room temperature, centrifuged (15,000 × g, 4 °C, 30 min), and the supernatant was analyzed for VFA by gas chromatography (Palmquist and Conrad, 1971).

Hand-plucked herbage was sampled at pre-grazing considering the grazing strata of each treatment in all grazing cycles (n = 25) (Congio et al., 2018). Concentrate samples were collected in five consecutive days at the end of each sampling period, bulked, and stored at room temperature for the chemical analysis.

Hand-plucked herbage, concentrate, and fecal samples were analyzed for DM, ash, ether extract (EE) (AOAC, 2005). The total N content was determined using an N analyzer, according to AOAC (1990), while NDF (Van Soest et al., 1991) was measured using sodium sulfite for all samples and heat-stable α-amylase for concentrate samples. Hand-plucked herbage samples were also analyzed for neutral detergent insoluble protein, acid detergent insoluble protein without Na_2SO_3 (Van Soest et al., 1991) and non-protein nitrogen (NPN) (Licitra et al., 1996). The following protein fractions were estimated: 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). Feed samples were also analyzed for acid-detergent fiber (ADF) and lignin (AOAC, 2005). The chemical composition of concentrate meal was: 88.4 % DM, 10.3 % ash, 14.0 % CP, 22.2 % NDF, 9.3 % ADF, 3.3 % EE and 49.8 % non-fibrous carbohydrate (NFC). The ingredients were: citrus-pulp (35 %), corn gluten feed (30 %), fine ground corn (20 %), soybean meal (10 %) and mineral (5 %). The concentrate was formulated using NRC (2001) considering forage CP level averaging 18 % DM for dairy cows yielding 20 kg d^{-1} of milk (similar to previous experiments in the same rotational grazing system; Voltolini et al., 2010; Danes et al., 2013; De Souza et al., 2017).

Milk samples were analyzed for fat, protein, lactose, total solids (TS), and milk urea nitrogen (MUN) using infrared procedures. Energy partitioning was calculated according to NRC (2001) as follows: Net energy (NE_L) intake (Mcal d^{-1}) = DMI (kg d^{-1}) × NE_L (diet) (Mcal d^{-1}); Milk NE_L (Mcal d^{-1}) = milk yield (kg) × [0.0929 × (fat %) + 0.0563 × (true protein %) + 0.0395 × (lactose %)]; NE_L BW gain = BW change (kg) × 5.65; NE_L activity (Mcal d^{-1}) = (4 × average distance between the milking center and paddocks (km d^{-1}) × 0.00045 (Mcal kg^{-1} km^{-1}) × BW (kg)) + (0.0012 × BW (kg)); NE_L available maintenance (Mcal d^{-1}) = NE_L (intake) – NE_L (BW gain) – NE_L (milk) – NE_L (activity). The efficiency of energy use was defined as NE_L (milk) / NE_L (intake) × 100. Fat corrected milk (3.5 %) (FCM; kg d^{-1}) was calculated as [(0.4324 × milk yield (kg)) + (16.216 × fat yield (kg))].

Statistical analysis

Statistical procedures were conducted using PROC MIXED in SAS (Statistical Analysis System, version 9.4). Different structures of the variance-covariance matrices were tested and the Bayesian Information Criterion was adopted to select the best-fit matrix. For herbage chemical composition analyses, paddocks were considered the experimental units, while cows were considered the experimental units for animal measurements. Blocks were considered random terms, and LI, sampling periods, and their interactions were treated as fixed effects. Sampling periods were analyzed as repeated measures. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Denominator degrees of freedom were adjusted by the Kenward-Rogers method and differences were declared significant at $p \leq 0.05$, while trends were declared at $p \leq 0.10$.

Results

The herbage CP content was greater for $LI_{95\%}$ than LI_{Max} ($p \leq 0.001$; Table 1). However, protein fractions, EE, and the NFC content were not influenced by grazing treatments ($p > 0.05$). There was interaction for treatment × period for CP, and for A + B1 and B2 protein fractions. Although there was no difference between treatments during P_1 (19.2 %; $p > 0.05$), herbage CP content was greater for $LI_{95\%}$ than LI_{Max} during P_2 (21.3 *vs.* 18.4 %; $p \leq 0.05$) and P_3 (22.7 *vs.* 20.6 %; $p \leq 0.05$). The A + B1 fraction was greater for LI_{Max} than $LI_{95\%}$ during P_1 (24.8 *vs.* 15.2 %; $p \leq 0.05$) with the inverse occurring in P_3 (36.0 *vs.* 25.8 %; $p \leq 0.05$). On the other hand, for the B2 fraction, $LI_{95\%}$ was greater during P_1 (47.2 *vs.* 34.0 %; $p \leq 0.05$) and lower during P_3 (24.9 *vs.* 40.2 %; $p \leq 0.05$).

Overall, cows grazing at $LI_{95\%}$ had greater intake than at LI_{Max} ($p \leq 0.05$; Table 2). Total DM, forage DM, OM, CP and NDF intakes were 15, 22, 20, 22 and 13 % greater ($p \leq 0.05$) for cows grazing at $LI_{95\%}$ than LI_{Max} (Table 2). Digestibility of DM, OM, CP and NDF as well as TTNDFD and pdNDF were also greater ($p \leq 0.05$) for cows grazing at $LI_{95\%}$ while no difference was detected for pdNDF kd ($p = 0.57$). There was interaction for treatment × period for pdNDF. During P_1, pdNDF was greater for cows grazing at $LI_{95\%}$ (73.3 *vs.* 68.0 %; $p \leq 0.05$), whereas there were no differences for P_2 (73.7 %; $p > 0.05$) and P_3 (73.5 %; $p > 0.05$).

Table 1 – Chemical composition (% of DM, unless specified otherwise) of hand-plucked herbage samples of elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}).

Item	Treatments		SEM	*p*-value		
	$LI_{95\%}$	LI_{Max}		Trt	Per	Trt × Per
OM	89.6	88.8	0.54	0.28	0.02	0.68
CP[1]	21.0	19.4	0.50	< 0.001	< 0.001	0.04
A + B1[2], % of CP	24.3	23.1	1.38	0.55	< 0.001	< 0.01
B2[2], % of CP	39.8	39.7	1.61	0.94	< 0.001	< 0.001
B3[2], % of CP	31.0	32.1	1.03	0.46	0.08	0.73
C[2], % of CP	5.4	6.0	0.59	0.54	0.04	0.30
EE	3.1	3.2	0.19	0.68	< 0.01	0.12
NFC	3.9	3.2	0.80	0.34	0.76	0.77

[1]Data from Congio et al. (2018). SEM = standard error of the mean; Trt = treatment; Per = period; DM = dry matter; OM = organic matter; CP = crude protein; [2]protein fractions adapted from Sniffen et al. (1992): A + B1 = non-protein nitrogen + rapidly degraded protein, B2 = protein with intermediate rates of degradation, B3 = slowly degraded protein and C = unavailable N; EE = ether extract; NFC = non-fibrous carbohydrate calculated as [100 – (NDF + ash + CP + EE)].

Cows grazing at $LI_{95\%}$ had greater fat corrected milk yield, milk lactose and MUN ($p \leq 0.05$) than cows grazing at LI_{Max} (Table 3). On the other hand, cows grazing at LI_{Max} had greater milk protein ($p \leq 0.01$) than at $LI_{95\%}$, whereas milk fat and BW change were not affected ($p > 0.05$) by the grazing treatments. There was interaction for treatment × period for MUN characterized by the absence of difference between treatments during P_1 (12.5 %; $p > 0.05$), and cows grazing at $LI_{95\%}$ presented greater MUN than LI_{Max} during both P_2 (13.1 *vs.* 11.9 %; $p \leq 0.05$) and P_3 (14.8 *vs.* 12.8 %; $p \leq 0.05$).

Cows grazing at $LI_{95\%}$ had greater concentration of total VFA, butyrate and valerate ($p \leq 0.05$) and smaller acetate ($p = 0.01$) than at LI_{Max} (Table 4). On the other hand, propionate, isobutyrate, isovalerate, acetate:propionate ratio, rumen microbial synthesis and microbial efficiency were not affected ($p > 0.05$) by the grazing treatments.

Intake of NE_L and its partitioning towards milk were greater ($p \leq 0.05$), however towards maintenance, it tended to be greater ($p = 0.07$) for cows grazing at $LI_{95\%}$ than at LI_{Max} (Table 5). The efficiency of energy use was greater for cows grazing at LI_{Max} than at $LI_{95\%}$ ($p = 0.03$).

Both urine and fecal N excretion were greater for cows grazing at $LI_{95\%}$ ($p \leq 0.05$) while NUE was not affected by the grazing treatments ($p = 0.54$; Table 6). The intensity of N excretion was smaller for cows grazing at $LI_{95\%}$ ($p = 0.03$).

Discussion

Congio et al. (2018) published results regarding herbage characteristics and animal responses, including milk yield and enteric methane emissions observed in this study. Overall, the $LI_{95\%}$ and LI_{Max} pre-grazing targets represented pre-grazing heights of 100 and 135 cm,

Table 2 – Intake and digestibility of dairy cows grazing elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}).

Item	Treatments		SEM	*p*-value		
	$LI_{95\%}$	LI_{Max}		Trt	Per	Trt × Per
Intake, kg d^{-1}						
Total DM[1]	18.2	15.9	0.61	< 0.01	0.11	0.22
Forage DM[1]	12.3	10.1	0.52	< 0.01	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	0.04	0.03	0.11
Digestibility, %						
DM	62.9	57.8	0.64	< 0.001	0.23	0.27
OM	67.1	60.6	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, % of total NDF	49.3	43.4	1.37	< 0.01	0.98	0.08
pdNDF, % of total NDF	75.5	70.2	0.86	< 0.01	0.44	0.01
pdNDF kd, % h^{-1}	3.6	3.4	0.27	0.57	0.69	0.99

[1]Data from Congio et al. (2018). SEM = standard error of the mean; Trt = treatment; Per = period; DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; TTNDF = predicted total–tract NDF digestibility; pdNDF = potentially digestible NDF; pdNDF kd = potentially digestible NDF fraction digestion rate.

Table 3 – Milk yield and composition of dairy cows grazing elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}).

Item	Treatments		SEM	*p*-value		
	$LI_{95\%}$	LI_{Max}		Trt	Per	Trt × Per
FCM, kg d^{-1}	18.4	16.9	1.18	0.02	0.02	0.63
Milk composition, %						
Fat	3.69	3.68	0.08	0.89	< 0.001	0.18
Protein	3.12	3.35	0.09	< 0.01	0.06	0.86
Lactose	4.49	4.38	0.04	< 0.01	0.02	0.96
MUN, mg dL^{-1}	13.4	12.2	0.42	< 0.01	< 0.01	0.03
BW change, kg[1]	0.44	0.55	0.36	0.61	< 0.01	0.61

[1]Data from Congio et al. (2018). SEM = standard error of the mean; Trt = treatment; Per = period; FCM = 3.5 % fat corrected milk calculated as [(0.4324 × milk yield) + (16.216 × fat yield)]; MUN = milk urea nitrogen; BW change = body weight change calculated as the difference in BW every sampling period.

Table 4 – Ruminal parameters of dairy cows grazing elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}).

Item	Treatments		SEM	*p*-value		
	$LI_{95\%}$	LI_{Max}		Trt	Per	Trt × Per
Total VFA, m*M*	64.99	55.19	3.403	0.04	0.01	0.08
VFA, mol 100 mol^{-1}						
Acetate	68.02	68.85	0.232	0.01	< 0.001	0.28
Propionate	16.90	16.53	0.202	0.20	< 0.001	0.97
Butyrate	11.44	10.87	0.127	< 0.01	0.06	0.95
Valerate	1.04	0.98	0.017	< 0.01	< 0.01	< 0.001
Isobutyrate	1.05	1.06	0.029	0.94	< 0.001	0.10
Isovalerate	1.53	1.47	0.061	0.47	< 0.001	0.04
Acetate: propionate ratio	4.04	4.18	0.062	0.12	< 0.001	0.88
Microbial synthesis, g d^{-1}	1312.3	1217.8	80.41	0.36	< 0.01	0.96
Microbial efficiency, g kg^{-1}	126.7	120.1	7.49	0.53	< 0.001	0.49

SEM = standard error of the mean; Trt = treatment; Per = period; VFA = volatile fat acids; Microbial efficiency = g per kg of digestible organic matter consumed.

Table 5 – Energy partitioning of dairy cows grazing elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}).

Item	Treatments		SEM	*p*-value		
	$LI_{95\%}$	LI_{Max}		Trt	Per	Trt × Per
NE_L intake, Mcal d^{-1}	25.4	20.4	0.95	< 0.01	0.33	0.01
Energy partitioning, Mcal d^{-1}						
NE_L maintenance	9.2	5.5	1.66	0.07	0.69	0.06
NE_L BW gain	3.4	3.1	1.57	0.87	< 0.01	0.63
NE_L activity	0.95	0.91	0.025	0.16	0.54	0.93
NE_L milk	12.1	11.2	0.58	0.05	0.06	0.92
Efficiency of energy use, %	50.4	58.1	3.18	0.03	0.75	0.06

SEM = standard error of the mean; Trt = treatment; Per = period; NE_L = net energy for lactation; BW = body weight.

Table 6 – Nitrogen partitioning of dairy cows grazing elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}).

Item	Treatments		SEM	*p*-value		
	$LI_{95\%}$	LI_{Max}		Trt	Per	Trt × Per
Urine N, g d^{-1}	201.7	180.6	9.56	0.04	< 0.01	0.69
Fecal N, g d^{-1}	155.3	142.0	5.01	0.03	0.98	0.65
NUE, %	16.8	16.1	0.99	0.54	< 0.001	0.87
N excretion intensity, g N kg milk^{-1}	19.9	21.9	1.18	0.03	< 0.001	0.59

SEM = standard error of the mean; Trt = treatment; Per = period; NUE = N-use efficiency.

respectively, whereas the post-grazing averaged 49.9 % of pre-grazing heights. Herbage accumulation was not affected by LI treatments; however, $LI_{95\ \%}$ accumulated more leaf (14,611 *vs.* 13,276 kg DM ha^{-1}) and less stem (795 *vs.* 3322 kg DM ha^{-1}) than LI_{Max}, whereas daily herbage allowance was greater for LI_{Max} than $LI_{95\%}$ (21.2 *vs.* 29.7 kg DM cow^{-1}). Grazing interval was shorter for $LI_{95\%}$ (21.1 *vs.* 31.7 days) and resulted in greater percentage of leaf in the pre-grazing herbage mass than LI_{Max} (95 *vs.* 81 %). As a result, hand-plucked herbage samples from $LI_{95\%}$ presented smaller contents of ADF (33.9 *vs.* 36.3 %) and lignin (3.3 *vs.* 3.8 %) and greater CP (21.0 *vs.* 19.4 %). Overall, the results regarding hand-plucked herbage chemical composition were similar to those from other studies conducted in the same site using similar N fertilization levels (Danes et al., 2013; De Souza et al., 2017). Increased N fertilization levels usually increases herbage CP content, due to greater proportions of soluble and intermediate protein fractions (Johnson et al., 2001). The average content of A + B1 + B2 protein fractions from hand-plucked samples was 63.5 %, in agreement with 66.4 % reported by Danes et al. (2013), also for elephant grass managed under a similar N fertilization level. The interaction of treatment × period observed for herbage CP characterized by the absence of difference between treatments during P_1 could be associated to the narrower (but significant) difference in the leaf-to-stem ratio between treatments compared to the other sampling periods (data not shown). In addition, differences in A + B1 and B2 protein fractions between treatments and sampling periods could be explained by the specific amounts of N fertilization applied in each treatment during each period (Johnson et al., 2001). The treatment × period interaction observed for herbage CP characterized by the absence of difference between treatments during P_1 and $LI_{95\%}$ greater than LI_{Max} in the other two sampling periods explains the same interaction observed for MUN.

Intake of grazing animals is strongly dependent on the sward structure (Hodgson, 1990). Based on the theoretical asymptotic curve described by Hodgson (1990), the greater intake of cows grazing at $LI_{95\%}$ is likely explained by the sward structural characteristics, such as smaller pre-grazing herbage mass and greater percentage of leaf in the grazing strata and their consequence on herbage preensibility and grazing behaviour of the animals. In a second phase, herbage chemical composition and the concentration of chemical compounds in the rumen play a more important role in controlling intake (Hodgson, 1990). The bulk of the results highlights the importance of the distribution and arrangement of aboveground plant parts in tropical pastures and reinforces the important role of sward structure in determining forage DMI of dairy cows. The smaller digestibility of nutrients in the herbage of the LI_{Max} treatment was associated to greater fiber content and its quality (*i.e.,* greater ADF and lignin), due to the longer grazing intervals and greater percentage of stem in the grazing strata compared to $LI_{95\%}$. On the other hand, greater fiber digestibility resulted in greater digestibility of both DM and OM, and VFA concentration with smaller proportion of acetate for cows grazing at $LI_{95\%}$. Ruminal fermentation of citrus pulp tends to produce relatively more acetate than propionate, compared to sugars and starch (Leiva et al., 2000). In this sense, the acetate-to-propionate ratio found in our study was higher than commonly found in studies that had starch as the main energy source in the concentrate (Danes et al., 2013). Leiva et al. (2000) reported that diets with greater amount of starch seemed to have NUE consistently higher than diets with neutral detergent-soluble fiber (*e.g.,* citrus pulp). However, the average NUE in our study (16.5 %) was similar to that reported by Danes et al. (2013) (16.2 %), who supplemented grazing cows with 6.1 kg of finely ground flint corn, suggesting no negative effect of citrus pulp inclusion on N metabolism.

The greater intake and digestibility of nutrients increased NE_L intake, allowing an increase by 8.9 % of FCM yield and greater milk NE_L from cows grazing at $LI_{95\%}$. These results show that it is possible to improve herbage quality resulting in greater DMI and FCM yield through good grazing management practices (pre-grazing target that minimized stem elongation and ensured leafy swards in our study, *i.e.,* $LI_{95\%}$), and corroborate the results of previous studies conducted in temperate swards (Wims et al., 2010; Muñoz et al., 2016).

Although FCM yield was higher, a greater proportion of dietary nutrients was used for maintenance of cows grazing at $LI_{95\%}$, resulting in lower efficiency of energy use. According to Geremia et al. (2014), pre-grazing target influences the grazing behaviour, with animals grazing at $LI_{95\%}$ having smaller bite mass and greater bite rate than at LI_{Max}. Since grazing determines additional requirements for maintenance, the tendency of greater maintenance requirements for cows grazing at $LI_{95\%}$ could be attributed to a more intense grazing activity to allow greater forage intake.

Both $LI_{95\%}$ and LI_{Max} hand-plucked herbage samples presented high CP content compared to pasture-based dairy operations in the tropics. This is partially associated to the 215 kg N ha^{-1} fractionally applied throughout experimental period. However, for the $LI_{95\%}$ target, the herbage CP content was further enhanced by the greater percentage of younger leaves in the herbage mass. Given that animals from both treatments were fed with concentrate at the same daily rate (5.9 kg, DM basis), the 22 % increase of CP intake of animals grazing at $LI_{95\%}$ was determined by both greater CP content of the herbage and forage intake. The greater CP intake of cows grazing at $LI_{95\%}$ did not affect their NUE, but it increased MUN and led to greater N losses through both urine and feces. As dietary CP content of $LI_{95\%}$ and LI_{Max} were 18.7 and 17.4 %, respectively, and 15.5 %, which were enough to meet the protein requirements of mid-lactation grazing cows (Danes et al., 2013), the energy required to process and excrete the N excess (*e.g.,* metabolic transformations, urea synthesis, and excretion by the kidneys) (Reed et al., 2017) may have increased maintenance requirements of cows from both treatments. The use of a concentrate with lower CP content might have allowed a better use of N from herbage, leading to better NUE for both treatments, as reported by Danes et al. (2013). However, greater N losses through excreta from cows grazing at $LI_{95\%}$ did not result in greater nitrous oxide emissions from the soil (Congio et al., 2019). In fact, there was mitigation of 40 % on nitrous oxide emission intensity from the soil when the $LI_{95\%}$ pre-grazing target was used (Congio et al., 2019). When the concept of emission intensity, usually used for methane emissions, is also utilized for N losses through excreta, the results show a 9 % reduction in N excretion intensity of dairy cows grazing at $LI_{95\%}$.

Conclusion

Strategic grazing management using the $LI_{95\%}$ pre-grazing target results in greater CP content of elephant grass and forage intake by dairy cows. On the other hand, it leads to greater CP intake and N losses through both urine and feces, and lower efficiency of energy use without affecting NUE by dairy cows. However, greater milk yield determines a 9 % reduction in N excretion intensity of dairy cows grazing at $LI_{95\%}$.

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