UNIVERSIDADE FEDERAL DE LAVRAS

PRISCILA JÚNIA RODRIGUES DA CRUZ

DEFOLIATION INTENSITY OF MARANDU PALISADE GRASS-PINTOI PEANUT MIXED PASTURE AFFECTS CANOPY STRUCTURE AND FORAGE INTAKE

LAVRAS-MG
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Prof. Dr. Daniel Rume Casagrande
Orientador

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APROVADA em 06 de dezembro de 2022

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“Somewhere, something incredible is waiting to be known.”

Carl Sagan

ABSTRACT

This dissertation aims to evaluate the effectiveness of clipping samples at stubble height to estimate the nutritive value of diets composed by marandu palisade grass and pintoi peanut grazed at three stubble heights; and to determine a defoliation intensity that would favor forage intake with more quality, increasing the apparent efficiency of nitrogen utilization. The trial was conducted at the Universidade Federal de Lavras, MG, Brazil for two consecutive years. The area was divided into 12 experimental units (paddocks) with three defoliation intensities (severe, moderate and light). The experimental area was constituted by marandu palisade grass (*Urochloa brizantha* cv. Marandu) and pintoi peanut (*Arachis pintoi* cv. Mandobi). In order to compare the nutritive value of samples from hand-plucking and clipping at stubble height, two hypotheses were tested: H_0: $\beta_0 = 0$ and H_0: $\beta_1 = 1$. Pearson's correlation analysis was performed to test if chemical composition of each methodology were correlated. The hypothesis H_0: $\beta_0 = 0$ was accepted for crude protein (CP), ash and protein-free neutral detergent fiber (NDF), and organic matter (OM) concentrations for marandu palisade grass and pintoi peanut. Hypothesis H_0: $\beta_1 = 1$ was rejected for CP, NDF, and OM concentrations for marandu palisade grass and for CP and OM of pintoi peanut ($P<0.01$), but not for NDF concentration of pintoi peanut ($P = 0.24$). The averages for CP, NDF, and OM for marandu palisade grass by hand-plucking were 7.9, 74.5, and 91.4 g/100 g DM and by clipping were 7.7, 68.7, and 90.7 g/100 g DM. For pintoi peanut the averages for CP, NDF, and OM by hand-plucking were 15.6, 63.5, and 92.0 g/100 g DM and by clipping were 15.9, 49.3, and 90.8 g/100 g DM. The Pearson's correlation coefficient was not significant for any chemical compound evaluated regardless the species; thus, the methodologies were not correlated ($P>0.05$). Defoliation intensity (DI), season (S) and the interaction between the two (DI×S) were considered fixed effects, year and replicate were considered random effects. Light defoliation showed greater forage allowance and lower stocking rate than the other intensities (6.3 kg DM/kg LW and 1.3 AU/ha). Grazing time did not differ in the first (390 min; P=0.15) or the last occupation day (440 min; P=0.20). During the last day, light defoliation showed 70% greater intake rate (33.2 g DM/min) than the average of the other treatments, related to greater biting rate (38.0 bites/min) and bite weight (0.9 g DM/bite). Grass and total dry matter intake (DMI; %LW) were greater for moderate and light defoliations, but legume intake was lower for severe and moderate defoliation. The efficiency of microbial synthesis (EMS) was 100 g of microbial protein/kg DOM greater during summer compared to spring but did not vary according to DI (P=0.80). Clipping mixed pasture at stubble height is not an efficient methodology to evaluate the nutritive value of the diet consumed because it is not capable of incorporating grazing selection, spatial and morphological heterogeneity. Moderate defoliation increases SR without reducing DMI.

Keywords: *Arachis pintoi*. Nitrogen balance. Forage intake. Nutritive value. *Urochloa brizantha*

RESUMO

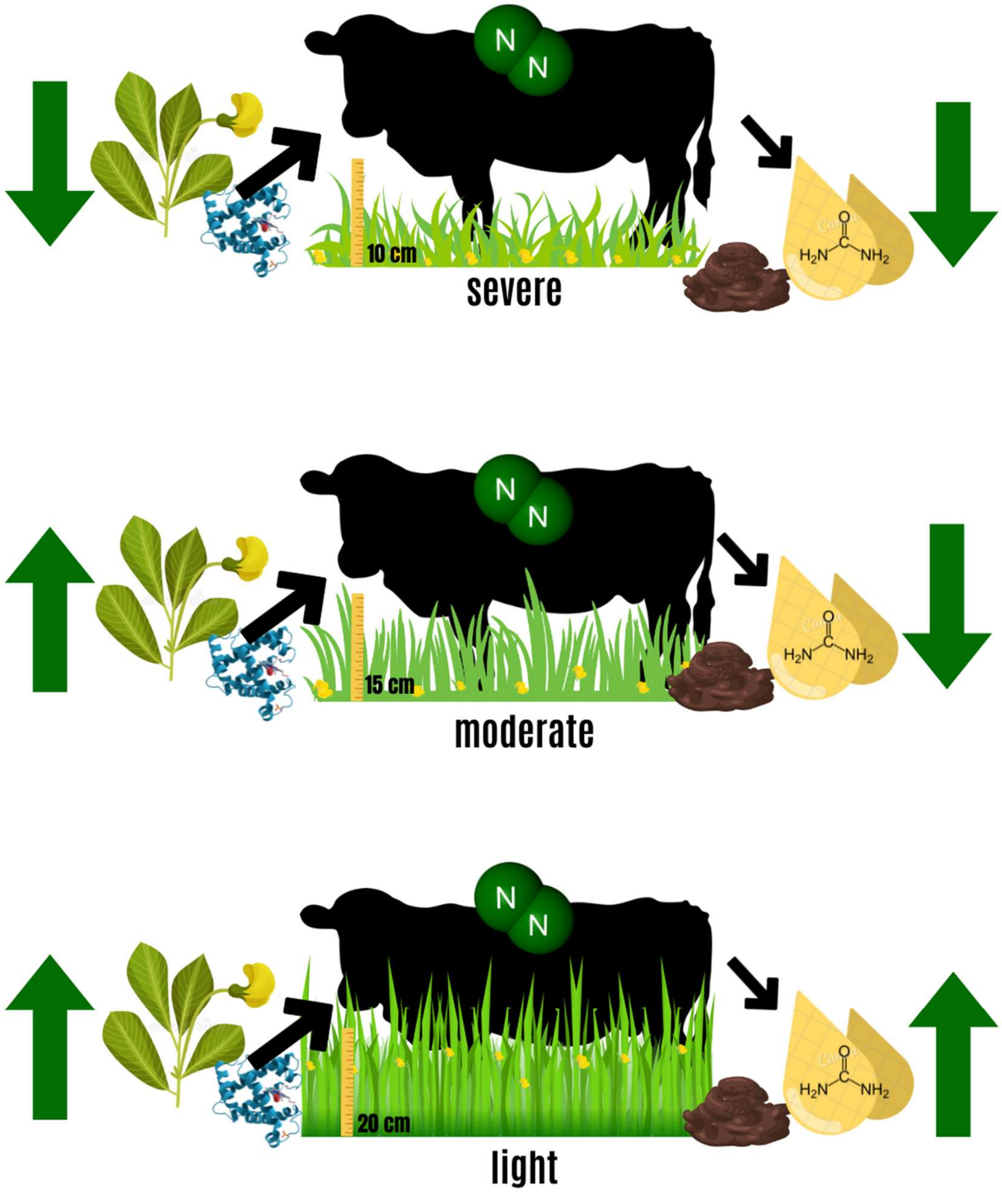
Essa tese tem como objetivo avaliar a efetividade do corte de amostras na altura de resíduo para estimar o valor nutritivo de dietas compostas de capim-marandu e amenodim forrageiro pastejados até três alturas de resíduo; e determinar a densidade de desfolhação que favorece o consumo de forragem de maior qualidade, aumentando a eficiência de utilização de nitrogênio aparente. O experimento foi conduzido na Universidade Federal de Lavras, MG, Brasil por dois anos consecutivos. A área foi dividida em 12 unidades experimentais (piquetes) com três intensidades de desfolhação (severa, moderada e leve). A área experimental era consituída de capim-marandu (*Urochloa brizantha* cv. Marandu) e amendoim forrageiro (*Arachis pintoi* cv. Mandobi). Para comparar o valor nutritivo de amostras coletadas por pastejo simulado e corte na altura do resíduo, foram testadas duas hipóteses: H_0: $\beta_0 = 0$ e H_0: $\beta_1 = 1$. Também foi realizada uma análise de correlação de Pearson para testar se a composição química de cada metodologia é correlacionada. A hipótese H_0: $\beta_0 = 0$ foi aceita para as concentrações de proteína bruta (PB), fibra em detergente neutro livre de cinzas e proteína (FDN) e matéria orgânica (MO) de capim-marandu e amendoim forrageiro. A hipótese H_0: $\beta_1 = 1$ foi rejeitada para as concentrações de PB, FDN e MO de capim-marandu, e para as concentrações de PB e MO de amendoim forrageiro ($P<0.01$), mas não para concetração de FDN do amendoim forrageiro ($P=0.24$). O coeficiente de correlação de Pearson não foi significativo para nenhum componente químico avaliado ($P>0.05$). Intensidade de desfolhação (ID), estação (E) e a interação entre intensidade de desfolhação e estação (ID×E) foram consideradas como efeito fixo, ano e repetição foram considerados como efeito aleatório. A desfolhação leve apresentou maior oferta de forragem e menor taxa de lotação que as outras intensidades (6.3 kg MS/kg PV e 1.3 UA/ha). Tempo de pastejo não diferiu no primeiro dia de ocupação (390 min; P=0.15) e nem no último dia (440 min; P=0.20). Durante o último dia, a desfolhação leve apresentou 70% maior taxa de ingestão (33,0 g MS/min) que a média dos outros tratamentos, relacionada com a maior taxa de bocados (38,0 bocados/min) e peso do bocado (0,9 g MS/bocado). O consumo total de forragem e de gramínea (CMS; %PV) foram maiores para desfolhações moderada e leve, mas o consumo de leguminosa foi menor para desfolhações moderada e severa. A eficiência de síntese de proteína microbiana (ESPM) foi 100 g de PM/kg MOD maior durante o verão comparado com a primavera, mas não variou de acordo com a ID (P=0.80). O corte de amostras de pastos mistos na altura do resíduo não é uma metodologia eficiente para avaliar o valor nutritivo de dietas consumidas porque não é capaz de incorporar o hábito de seleção e as heterogeneidades espaciais e morfológicas. A desfolhação moderada aumenta a taxa de lotação sem reduzir o CMS.

Palavras-chave: *Arachis pintoi*. Balanço de nitrogênio. Consumo de forragem. Valor nutritivo. *Urochloa brizantha*

INTERPETATIVE SUMMARY

Adding legume forages in a monoculture pasture show many benefits for the system, such as providing nitrogen (N) to the grass through nitrogen biological fixation (NBF) or decomposition of shoots and roots that show greater N content than grasses; improving nutritive value of the diet due to greater protein concentration and less neutral detergent fiber (NDF) than grasses; providing ecosystem services due to the variation on pasture botanical composition and diversifying the system. Mixed pastures between legume forages and grasses have been used worldwide for many years, mainly in temperate regions. In Brazil, for many years there was a belief that legumes were not physio and morphologically compatible with tropical grasses and mixed pastures were not stable and persistent; what has been proven wrong with many studies carried on along the past years. Legume forages that present a stoloniferous growing habit, such as the forage peanut, tend to be more persistent in a mixed pasture due to its capacity of rooting when its stolons are close to the ground and be independent of the main axis, thus some growing points can be consumed or die, and the young stolons would continue alive. The benefits brought by the legume and its persistence will depend on its proportion in total herbage mass and its proportion will depend mainly on the defoliation management; how much of forage its being removed and in which frequency. Some studies indicate that less intense and more frequent grazing cycles would reduce the legume forage but would increase forage intake and increase the metabolism of N, thus losing less N to the system. In this study we evaluated three defoliation regimes: severe (10 cm of stubble height), moderate (15 cm), and light (20 cm). The grazing was made by at least two beef heifers for nine days, and more heifers were added when necessary to obtain the desirable stubble height. Light defoliation led to greater forage allowance which increased short-term intake, greater grass and legume intake, and less N excretion per hectare even though there was more N excretion by feces for this defoliation regime. However, to recommend a defoliation regime, it must be considered not only animal responses but also the whole system. Therefore, the moderate defoliation showed similar forage intake but increased stocking rate, leading to an intermediate N excretion per hectare.

GRAPHICAL ABSTRACT



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GENERAL INTRODUCTION

Mixing legume forages with grasses brings many benefits for grazing systems in tropical regions, such as increasing nutritive value of the diet leading to greater performance (Homem et al., 2021; dos Santos et al., 2022), increasing total forage production by providing nitrogen for the grass through N biological fixation (Depablos et al., 2021; Garcia et al., 2021; Jaramillo et al., 2021a), and greater nutrient cycling and herbage production by improving litter quality (Vendramini et al., 2014; Menezes et al., 2015; Gomes et al., 2020). Despite the many benefits, the use of legume forages in tropical regions is still below its use in temperate regions, mainly associated to difficulties for establishment, low productivity and persistence (Muir et al., 2014; Menezes et al., 2015). To achieve the success of a stable mixed pasture, the first step is to select species and cultivars adapted to edaphic and environmental conditions and resistant to pest and diseases (Thomas, 1995). Legumes present indeed, lower dry matter production compared to tropical grasses (Gomes et al., 2018; Homem et al., 2021; dos Santos et al., 2022) and slower establishment. However, the compatibility between grasses and legumes and its persistence on the pasture depends mainly on the propagation mechanism and light management (Homem et al., 2019; Boddey et al., 2020).

Pintoi peanut (*Arachis pintoi*) develops stolons and can increase its population through vegetative propagation. This form of growth makes the pintoi peanut one of the most compatible legumes in mixed pastures, because it allows decapitation or death of main axis, but rooted young stolons can survive (Boddey et al., 2020). The benefits of including a legume forage in the system and its persistence will depend on the proportion of legume (Thomas, 1995). Thomas (1992) indicates that the legume must have between 20 to 45% on the total dry matter herbage mass in order to provide nitrogen for a productive and sustainable pasture; and between 20 to 30% to meet the optimal for animal production. The proportion of legume and its persistence will depend on the defoliation management, which could be defined as the removal of part of shoot organs, and it is characterized by intensity and frequency (Gastal and Lemaire, 2015; Tamele et al., 2018; Homem et al., 2019).

Under continuous stocking Tamele et al. (2018) showed greater proportion of pintoi peanut in the upper strata when the canopy was managed between 30 and 40 cm, consequently, there was lower stolon density and proportion of legume in the forage mass. Regarding the intermittent stocking, Gomes et al. (2018) and Pereira et al. (2017) evaluated four light interceptions for mixed pastures of marandu palisade grass and pintoi peanut, between 87 and 100%. Both studies showed a decrease in stolon density and legume mass under 100% light interception, when the canopy height was around 38 cm.

The pintoi peanut shows greater crude protein and lower neutral detergent fiber concentrations than tropical grasses (Gomes et al., 2018; Homem et al., 2021; Jaramillo et al., 2021b), which can improve the nutritive value of the diet leading to greater performance. It is crucial to correctly determine the nutritive value of the diet in mixed pastures, once it would help to choose the best supplementation and management strategy aiming to obtain greater animal performance and productivity.

There are two common methodologies to assess the nutritive value of a diet. The most used is the hand-plucking, which consists of following and observing the grazing animal, and trying to collect the forage similar to what the animal is grazing (de Vries, 1995). The methodology requires calibration between the person and the animal; besides it is necessary to identify the species grazed and the amount of each, what could be challenging. The second methodology consists of clipping the forage at the stubble height, assuming that the animal is consuming all the forage above that stratum, however it does not consider the animal selective ability. All methodologies show pros and cons, it is necessary to determine which one corresponds more to the real diet and its applicability for research and at field level.

This dissertation aims to compare the nutritive value of marandu palisade grass and pintoi peanut collected by hand-plucking or clipping at stubble height; and, to determine a defoliation intensity that would favor forage intake with more quality, increasing the apparent efficiency of nitrogen utilization in the animal's body.

CHAPTER 1

Article 1 – Can clipping at stubble height be an alternative to estimate nutritive value of mixed pastures?

Abstract

Correctly assess the nutritive value of the diet in mixed pastures would help to choose the best supplementation and management strategy leading to greater animal performance and productivity. The aim of this study was to evaluate if clipping forage at stubble height would represent diets composed by palisadegrass and pintoi peanut compared to samples collected by hand-plucking. The trial was carried out at the Universidade Federal de Lavras, MG, Brazil for two consecutive years. The area was divided into 12 experimental units (paddocks) with three defoliation intensities: severe (10 cm of stubble height), moderate (15 cm of stubble height) and light (20 cm of stubble height). The experimental area was constituted by marandu palisade grass (*Urochloa brizantha* cv. Marandu) and pintoi peanut (*Arachis pintoi* cv. Mandobi). The occupation period of the rotational mob stocking started when the canopy reached 24 to 27 cm and lasted for 9 days. Two Tabapua heifers were used per treatment as testers and put-and-take animals were added when it was necessary to achieve the planned stubble height for each treatment. In order to compare the nutritive value of samples from hand-plucking and clipping at stubble height, two hypotheses were tested: H_0: $\beta_0 = 0$ and H_0: $\beta_1 = 1$. Pearson's correlation analysis was performed to test if chemical composition of each methodology were correlated. The hypothesis H_0: $\beta_0 = 0$ was accepted for crude protein (CP), ash and protein-free neutral detergent fiber (NDF), and organic matter (OM) concentrations for marandu palisade grass and pintoi peanut. Hypothesis H_0: $\beta_1 = 1$ was rejected for CP, NDF, and OM concentrations for marandu palisade grass and for CP and OM of pintoi peanut ($P<0.01$), but not for NDF concentration of pintoi peanut ($P = 0.24$). The averages for CP, NDF, and OM for marandu palisade grass by hand-plucking were 7.9, 74.5, and 91.4 g/100 g DM and by clipping were 7.7, 68.7, and 90.7 g/100 g DM. For pintoi peanut the averages for CP, NDF, and OM by hand-plucking were 15.6, 63.5, and 92.0 g/100 g DM and by clipping were 15.9, 49.3, and 90.8 g/100 g DM. The Pearson's correlation coefficient was not significant for any chemical compound evaluated regardless the species; thus, the methodologies were not correlated ($P>0.05$). Clipping mixed pasture at stubble height is not an efficient methodology to evaluate the nutritive value of the diet consumed because it is not capable of incorporating grazing selection, spatial and morphological heterogeneity.

Keywords: hand-plucking, grazed strata, forage intake, chemical composition, legume forage

1. INTRODUCTION

Ruminants grazing mixed pastures face many choices, including where and when to graze and how much forage to consume, which affects not only the nutritional status of the animal, but also the canopy composition and the nutritive value based on selective defoliation (Soder et al., 2009). The nutritive value of a diet can be defined as the predicted animal response based on chemical composition, digestibility and nature of digested products (Allen et al., 2011). Tropical grasses usually present lower nutritive value than legumes and temperate grasses, and it may be affected by the climate, region, and management practices (Oelberg, 1956; Gomes et al., 2018; Lee, 2018; Homem et al., 2021). In tropical areas, an interesting option to increase the nutritive value of a diet without increasing costs substantially, is to grow grasses mixed with forage legumes. This practice has shown to increase about 2 percentual units in crude protein concentration compared to pastures without legumes or fertilizing (Homem et al., 2021; dos Santos et al., 2022). Thus, it is crucial to measure correctly the nutritive value of a diet of animals raised over pastures in order to optimize pasture and cattle management.

The most common methodology used to determine the nutritive value of a diet is the hand-plucking (de Vries, 1995). This method is described as fast, cheap, and simple, consisting in the observation of animals' grazing behavior prior to collecting the samples. For this method, samples are plucked between the thumb and a backward-bent forefinger, simulating the grazing behavior (de Vries, 1995). This methodology is used as a standard for the most grazing studies, both for mixed pasture and pure stand. Moreover, it relies on a calibration between the person who is collecting and the animal. Besides, in mixed pastures it is necessary to identify the forage species consumed and to quantify how much of each plant was consumed (Papachriston et al., 2005).

The second methodology frequently used is to clip forages at stubble height, assuming that the animal is consuming everything above that point. Therefore, this approach also does not consider the selective ability of grazing cattle, which will be more selective when forage is available and more heterogeneous (Culley et al., 1933; Hughes et al., 2010).

Hughes et al. (2010) investigating the nutritive value of bahiagrass by hand-sampling and masticate-sampling reported that masticated samples presented greater crude protein concentration and greater in vitro digestible organic matter. So, when animals can select, they will choose species and plant components that have greater nutritive value, which could be underestimated by hand-sampling.

Clipping at stubble height would be an easy methodology to apply in mixed pastures, once it does not need the observation of animals' behavior. In addition, proper evaluation of the diet nutritive value is necessary to identify possible nutritional deficiencies and to formulate supplementation strategies to improve animal performance.

The objective of this study was to evaluate if clipping the forage at stubble height would represent the nutritive value of the consumed diet in a mixed pasture and compare with hand-plucking samples, as a standard. We hypothesize that nutritive value of samples collected by clipping at stubble height could be similar to hand-plucking due to its collection on grazed strata level.

2. MATERIAL AND METHODS

All experimental procedures were approved by the Ethics and Animal Welfare Committee of the Federal University of Lavras (protocol number 026/2019).

a. Experimental site

The study was conducted at the Experimental Farm of the Federal University of Lavras, Brazil (21°14′S, 45°00′W; 918 m above sea level). The area presents a subtropical humid mesothermal climate with dry winters (Köppen-Geiger climate classification: Cwa; (de Sá Júnior et al., 2012). Meteorological data were obtained from a weather station located 1,000 m from the experimental area (Fig. 1).

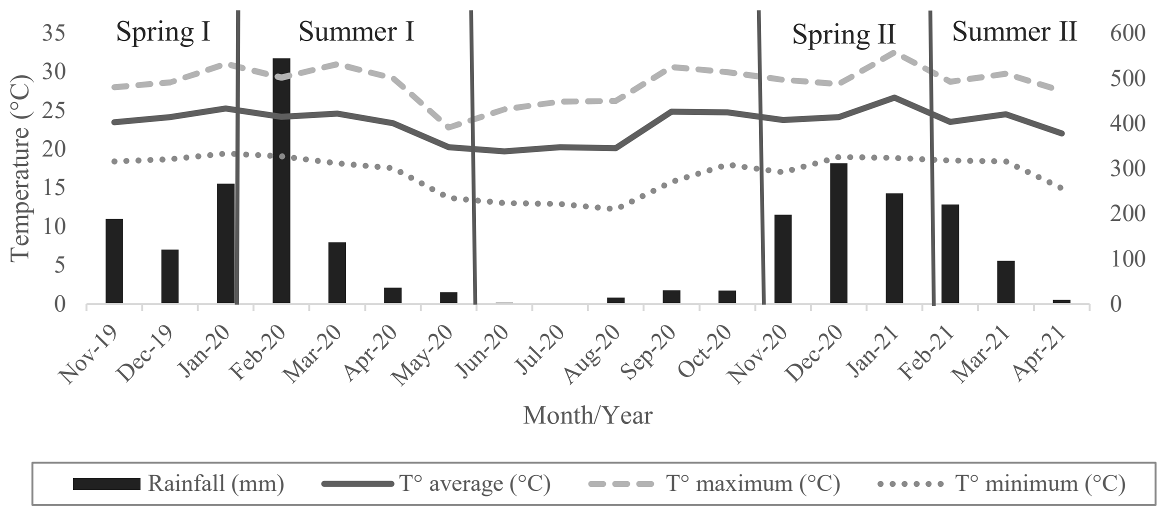
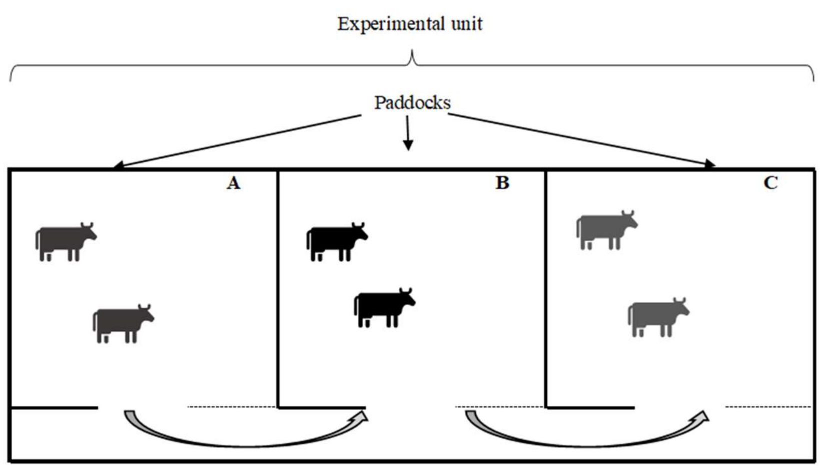


Figure 1. Monthly temperatures (°C) and rainfall (mm) in Lavras, Brazil, during the experimental period (seasons of the year).

The pasture used was established in December 2006 by joint seeding of marandu palisade grass (*Urochloa brizantha* syn. *Brachiaria brizantha* cv. Marandu) with pintoi peanut (*A. pintoi* cv. BRS Mandobi). The seeding rates were 7.6 and 7.2 kg/ha of pure live seed for palisade grass and pintoi peanut, respectively.

The soil of the experimental site at the start of the study (November 2019) were pH = 5.6, OM = 4.58 dag/kg, P = 2.82 mg/dm^3, P-rem= 24.8 mg/l, K^+ = 69.41 mg/dm^3, Ca^{2+} = 1.91 cmol$_c$/dm^3, Mg^{2+} = 0.52 cmol$_c$/dm^3, H + Al = 3.1 cmol$_c$/dm^3, Al^{3+} = 0.09 cmol$_c$/dm^3, and cation exchange capacity = 5.71 cmol$_c$/dm^3. Fertilizers were applied as single superphosphate and potassium chloride. Applications (kg/ha) corresponded to 40 of P_2O_5, 53 of K_2O, according to the soil analysis. Thirty days before starting the experiment, it was also applied agricultural gypsum (1.39 t/ha) and limestone (278 kg/ha).

The pasture was divided into 12 experimental units (paddocks) and each paddock was subdivided into three paddocks of the same size (A, B, and C). The area of each treatment considered an occupation period of nine days by two animals weighing an average of 250 kg and consuming 2% of live weight (Fig. 2). Thus, each replicate of light defoliation had 1080 m^2, moderate had 660 m^2, and intense had 480 m^2. A 2-yr experimental period (from 2019 to 2021) was adopted, from November to January (Spring) and from January to April (Summer; Fig. 1). Assessments were not performed from May to September for both years due to the climatic conditions (dry winter).



Item	Period of ocupation (d)								
	1	2	3	4	5	6	7	8	9
Hand-plucking sampling							x		x
Clipping at stubble height							x		

Figure 2. The experimental unit was divided into three paddocks with 3-d occupation period in each one, totaling 9 days. A reserve mixed pasture of palisade grass and pintoi peanut was used to keep animals during rest periods. All the evaluations were performed as explained in the figure.

b. Experimental management

The grazing managements strategies consisted of three defoliation intensities (DI): severe (10 cm), moderate (15 cm) and light (20 cm of stubble height). The treatments were disposed on a completely randomized design with four replicates, equaling 12 experimental units. The rest period length was determined as a function of each treatment. Therefore, for the severe defoliation intensity it was necessary a longer rest period. All paddocks started being grazed when the canopy reached between 24 and 27 cm of height (Gomes et al., 2018). A minimum of two Tabapua heifers (265 ± 20 kg) were used to graze paddocks to the target stubble height for each treatment.

Additional put-and-take heifers were added to make the canopy achieve the treatment height during a 3-d period.

c. Experimental evaluations

Nutritive value

Hand-plucking samples were collected for forage nutritive value analysis (de Vries, 1995). Samples from the mixture were collected and separated in grass and legume in the lab before drying. Samples from the first and the last day of occupation for each species were analyzed separated.

Samples from clipping at stubble height for marandu palisade grass and pintoi peanut were collected only on the first occupation day according to the defoliation management. Samples were collected above 10, 15, and 20 cm. Grass and legume were analyzed separated.

All the samples from grazed strata, ruminal extrusa, and hand-plucked were oven-dried at 55 °C for 72 h, and ground in a Cyclotec mill (Tecator, Herndon, VA) to pass a 1-mm screen. The DM of each sample was obtained by oven drying at 105 °C for 16 h (INCT-CA G-003/1; Detmann et al., 2012). Ash concentration was determined by 4-h incineration process in a 600 °C muffle furnace (method INCT-CA M-001/1; Detmann et al., 2012). The crude protein (CP) concentration was calculated based on the N concentration (CP = total N × 6.25), which was determined using the Kjeldahl procedure (method INCT-CA N-001/1; Detmann et al., 2012). The neutral detergent fiber (NDF) was determined by the autoclave method at 105°C for 60 min (method INCT-CA F-002/1; Detmann et al., 2012). The ash and protein-free NDF was obtained by using sodium sulfite and incineration (Robertson, 1981).

d. Statistical analysis

The nutritive value of marandu palisade grass and pintoi peanut by hand-plucking was calculated as the average between the first and the last day of occupation.

The models consisted of fitting a first-order equation of the type $Y = \beta_0 + \beta_1 x$ with the following hypotheses: H_0: $\beta_0 = 0$ and H_0: $\beta_1 = 1$. Where Y represented the nutritive value of samples collected by hand-plucking and X represented samples collected by clipping at stubble height. Data were analyzed using the REG procedure by SAS (SAS Institute Inc.). Pearson's correlation analysis was performed between methodologies for the same variable using PROC CORR, also by SAS. Differences were stated at $P \leq 0.05$.

3. RESULTS

Comparison between methodologies and descriptive statistics are presented in Table 1. Hypothesis H_0: $\beta_0 = 0$ was accepted for crude protein (CP), ash and protein-free neutral detergent fiber (NDF), and organic matter (OM) concentrations of marandu palisade grass and for pintoi peanut (P>0.05). Hypothesis H_0: $\beta_1 = 1$ was rejected for CP, NDF, and OM for marandu palisade grass and for CP and OM of pintoi peanut (P<0.01), but not for NDF concentration of pintoi peanut (P=0.24). Rejecting H_0: $\beta_1 = 1$ means that there are bias to the equation, which over or underestimate the values of nutritive value.

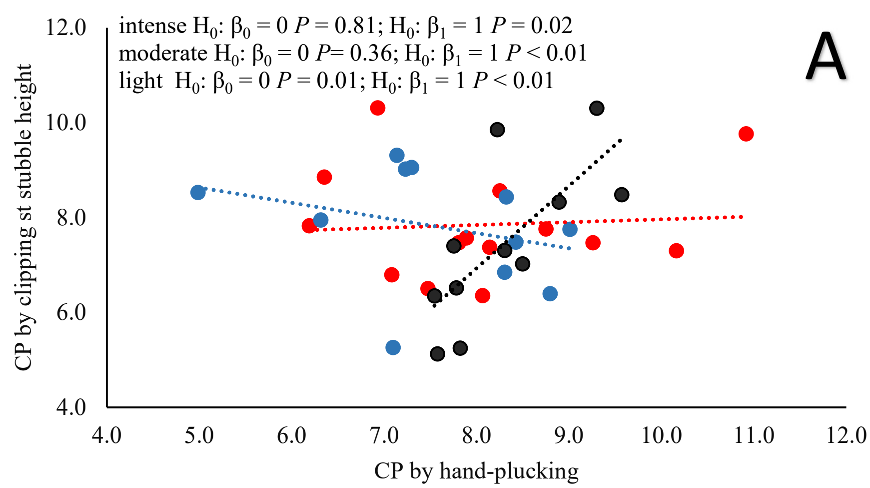
Table 1. Comparison between the nutritive value of hand-plucking samples and clipping at stubble height for marandu palisade grass and pintoi peanut

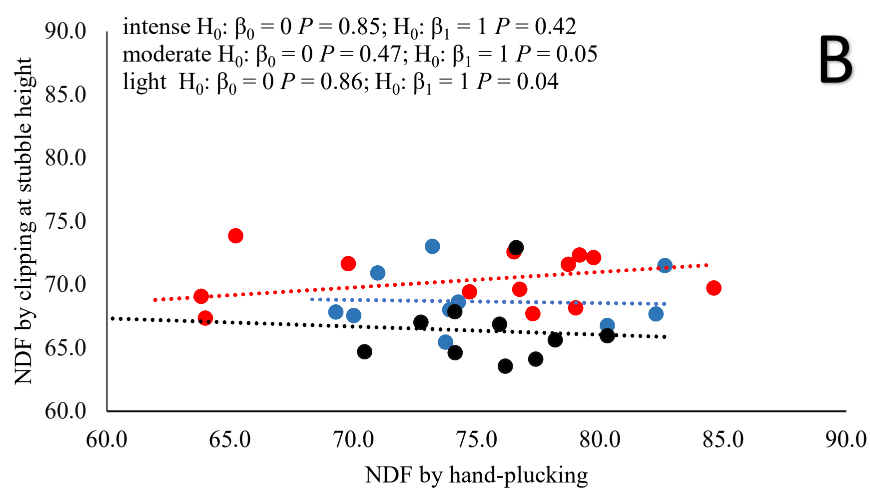
	hand-plucking				clipping at stubble height				P-value	
	mean	**SD**	**minimum**	**maximum**	**mean**	**SD**	**minimum**	**maximum**	**H_0: $\beta_0 = 0$**	**H_0: $\beta_1 = 1$**
marandu palisade grass (g/100 g DM)										
CP	7.9	1.2	5.0	10.9	7.7	1.3	5.1	10.3	0.67	<0.01
NDF	74.5	5.9	54.4	84.6	68.7	2.8	63.5	73.8	0.78	<0.01
OM	91.4	0.8	90.0	94.1	90.7	0.9	88.2	93.1	0.30	<0.01
pintoi peanut										
CP	15.6	1.8	11.8	19.6	15.9	2.1	10.5	19.9	0.70	<0.01
NDF	63.5	8.1	50.0	80.3	49.3	4.0	42.3	58.5	0.07	0.24
OM	92.0	0.8	90.0	93.0	90.8	1.0	87.4	92.7	0.94	<0.01

Abbreviations: CP = crude protein; NDF = ash and protein-free neutral detergent fiber; OM = organic matter. SD = standard deviation; P-values were significant when P ≤ 0.05.

Comparison of CP, NDF, and OM concentrations of marandu palisade grass for three defoliation managements are shown in the Figure 3A, B, and C. There was no evidence to reject the hypothesis H_0: $\beta_0 = 0$ (P>0.05) for CP concentration of marandu palisade grass under intense and light defoliation, however H_0: $\beta_1 = 1$ was rejected (P<0.01). Under light defoliation, both hypotheses were rejected (P>0.05). The hypothesis H_0: $\beta_0 = 0$ was accepted for NDF concentration regardless the defoliation intensity (P>0.05), but only for intense defoliation the hypothesis H_0: $\beta_1 = 1$ was accepted (P=0.42). Thus, the methodologies did not differ for NDF concentration only for intense defoliation. The comparison between methodologies for pintoi peanut nutritive value in each defoliation management is shown in Figure 4A, B, and C. The hypothesis H_0: $\beta = 0$ cannot be rejected for CP, NDF, and OM regardless the defoliation intensity (P > 0.05). In the three defoliation intensities, for CP and OM concentrations, the hypothesis H_0: $\beta_1 = 1$ was rejected

(P<0.01), but not for NDF concentration (P>0.05). Thus, the methodologies are similar only for NDF concentration along the three intensities.





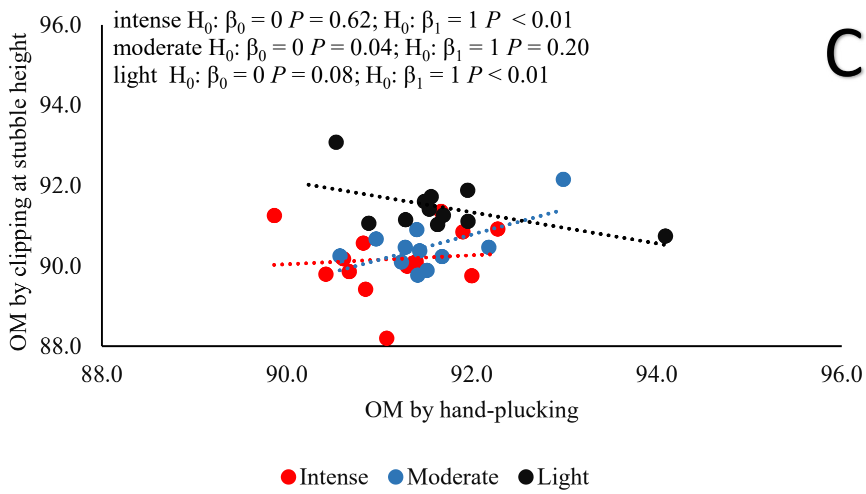
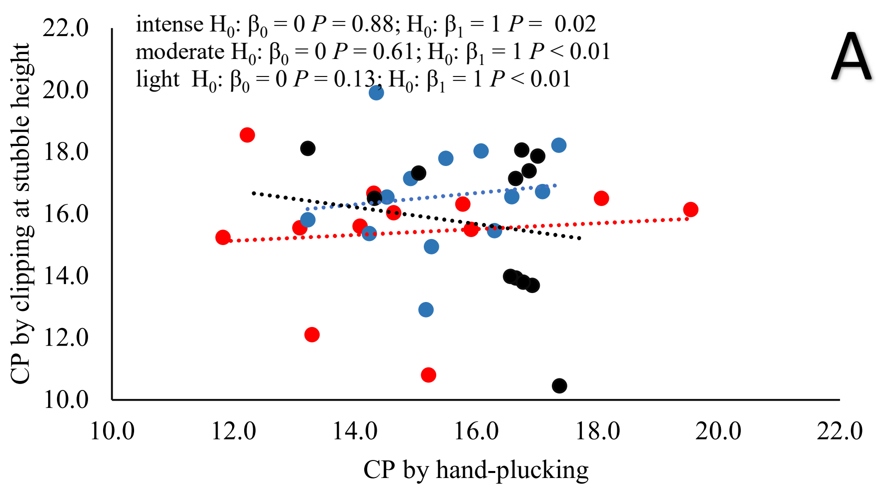


Figure 3. Comparison between nutritive value of marandu palisade grass for hand-plucking and clipping at stubble height samples collected at three defoliation managements



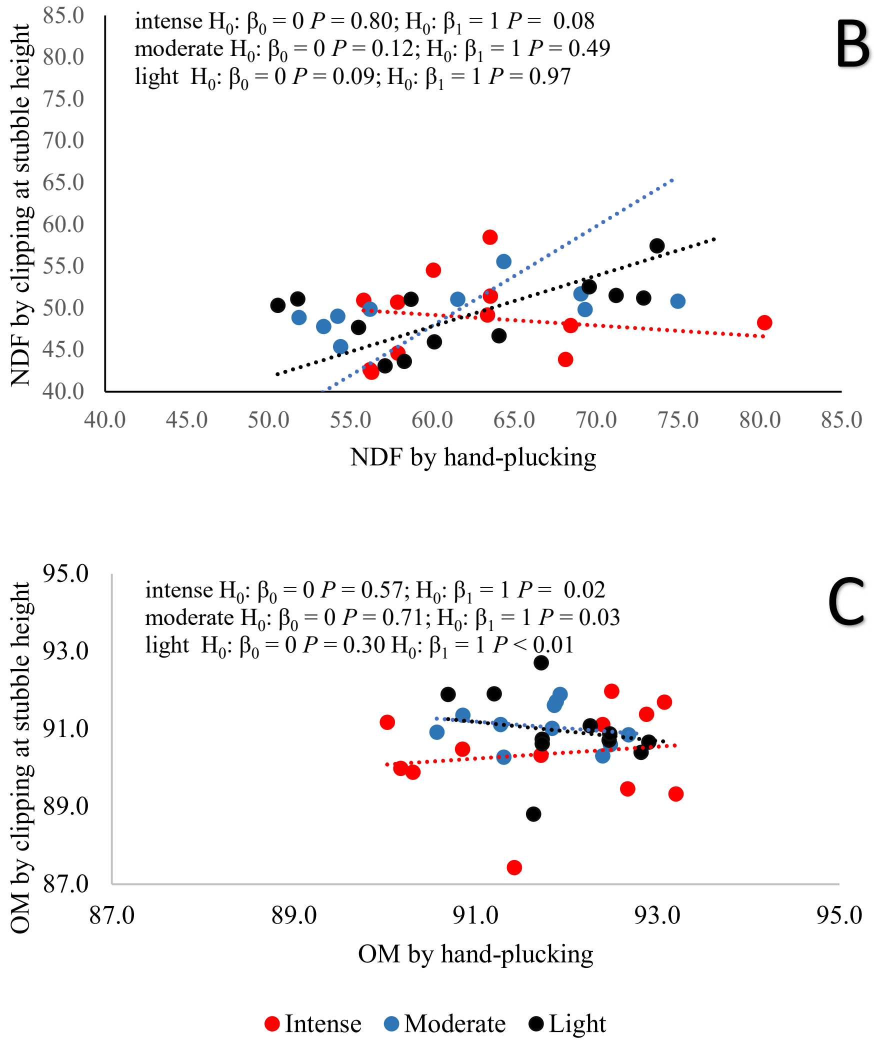


Figure 4. Comparison between nutritive value of pintoi peanut for hand-plucking and clipping at stubble height samples collected at three defoliation managements

The nutritive value of marandu palisade grass and pintoi peanut collected by hand-plucking did not show correlation with samples collected by clipping at stubble height (Table 2; P>0.05).

Table 2. Pearson's correlation coefficient for nutritive value of marandu palisade grass and pintoi peanut evaluated by hand-plucking and clipping at stubble height

	marandu palisade grass			**pintoi peanut**		
	CP	**NDF**	**OM**	**CP**	**NDF**	**OM**
Pearson correlation coefficient	0.08	-0.01	0.18	-0.06	0.29	0.01
P-value	0.66	0.94	0.29	0.72	0.08	0.94

Abbreviations: CP = crude protein; NDF = ash and protein-free neutral detergent fiber; OM = organic matter. P-values were significant when $P \leq 0.05$.

4. DISCUSSION

When a pasture is partially grazed, under light defoliation, the utilization by livestock is very irregular, with areas grazed to within few centimeters from the soil surface and other areas hardly touched. Another difference is when there are more species composing the canopy, one or more may be consumed closed to the soil surface, and other species are eaten only the top layer (Culley et al., 1933). Considering these two factors, we aimed to compare the effectiveness of clipping at stubble height to determine the nutritive value of mixed pastures under three defoliation intensities.

The correct measure of nutritive value of a diet is essential for estimating daily intake of each nutrient, digestibility, performance, and make correctly adjustments according to cattle requirements, even to increase stocking rate (Smith et al., 2020). Several experiments have been developed in order to compare hand-plucking to the actual diet consumed by the animal (Edlefsen et al., 1960; Kiesling and Nelson, 1969; de Vries, 1995). Most of them compare samples collected by hand-plucking with ruminal or esophageal extrusa, since extrusa represents the selection capacity of the animal. Edlefsen et al. (1960) compared the nutritive value of samples collected by esophageal extrusa of sheep grazing mixed pastures and pure stands. The authors reported statistical differences between samples from extrusa and hand-plucking for five of eight chemical compounds evaluated, the differences were primarily associated to saliva contamination. Thus, they state that due to the small differences, hand-plucking could be considered an effective methodology to estimate the diet consumed. de Vries (1995) also compared the effectivity of hand-plucking for different vegetation types and showed that the regression intercept was not different from zero and the regression coefficients were not different from unit. Therefore, the estimate of nutritive value by hand-plucking showed no significant bias. The main challenge to the method is the adjustment between animals and operators, so the operator does not disturb their natural behavior.

Hand-plucking has been used as a standard method for evaluating nutritive value of forages, as its evaluation with fistulated animals is expensive, laborious, and time consuming, besides the concern about animal welfare. The grazing preferences of animal changes along the day in response of internal and external influences and visiting animals, such as the rumen cannulated may not select the same material as resident animals; besides, samples of hand-plucking are free from salivary contamination (Coates et al., 1987; de Vries, 1995). Therefore, hand-plucking could be considered the methodology that most represent the nutritive value of a diet consumed.

We assumed the nutritive value based on hand-plucking samples was the standard and tested the hypothesis H0: $\beta_0 = 0$, in order to prove that when the variables of nutritive value were zero for hand-plucking, it would be zero for clipping at stubble height (Table 1). The hypothesis was not rejected for CP, NDF, and OM measured for marandu palisade grass and pintoi peanut. However, in order to test the bias between data obtained by the methodologies, we tested H_0: $\beta_1 = 1$. Not including pinto peanut NDF concentration, we could reject H0: β1 = 1 for all the variables evaluated, thus there are some bias and errors inherent of clipping methodology causing differences between data from each methodology. These results corroborate the Table 2, that shows the absence of correlation between methodologies for all variables evaluated ($P>0.05$). The methodologies could be considered similar for NDF concentration of palisade grass under intense defoliation and NDF concentration of pintoi peanut regardless the defoliation (Figures 3B and 4B). Nonetheless, the methodology used to define nutritive value should be effective for all botanical and chemical component and defoliation intensity.

The main difference between clipping and hand-plucking is the manner in which the forage is being removed. In clipping, the forage is cut uniformly at a given height, while hand-plucking simulated the removal by the animals by pulling and breaking the shoot in a convenient height, which varies according to species of forage or classes of livestock (Culley et al., 1933).

Comparing clipping samples with extrusa, Jefferies and Rice (1969) reported differences between methodologies along the year and showed that clipping samples usually underestimate the diet nutritive value because it does not consider grazing habits and preferences. Sankhyan et al. (1999) evaluated the nutritive value of buffel-grass (*Cenchrus ciliaris*) according to season and methodology. Clipping showed lesser CP and greater NDF concentrations than hand-plucking and extrusa, regardless the season. Clipping overestimated dry matter intake in this study.

Muir et al. (2008) compared two clipping heights (7.5 and 15 cm) and hand-plucking to evaluate the nutritive value of several annual warm-season legume forages. The authors found lower CP concentration for samples collected by stubble height of 7.5 and 15 cm compared to hand-plucking. Greater nutritive value was reported by Butler et al. (2007) for rhizoma peanut collected by hand-plucking compared to clipping samples above 5 and 10 cm. The nutritive value of leaves is greater than steams and dead material, so samples from hand-plucking containing mostly leaves should have greater nutritive value than clipped samples containing steams and dead material (Goes et al., 2003; Muir et al., 2008). Another difference is that animal preference is not represented by clipping studies, particularly if more than one species is present (Culley et al., 1933).

5. CONCLUSION

The nutritive value of a pasture consists of its botanical and chemical composition, digestibility, canopy structure, and intake. Those factors are directly dependent of animal grazing behavior and selectivity and should be considered as taking samples to evaluate nutritive value. Clipping at the stubble height is not an alternative to measure the nutritive value of consumed diet of mixed pasture. The best approach to estimate the nutritive value of a diet in a mixed pasture is the hand-plucking, due to the low cost and labor, and the capacity of incorporating grazing selection by animals. Hand-plucking must be calibrated in order to accurately represent differences between heterogeneous pastures.

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CHAPTER 2

Article 2 – Effect of post-grazing canopy height on canopy structure, ingestive behavior, dry matter intake, and nitrogen metabolism in beef heifers grazing mixed pastures of palisade grass-pintoi peanut

Abstract

Higher mixed canopies tend to reduce the legume proportion due to etiolation of pintoi peanut and exposure of growing points to animal removal, leading to a lower stolon density, reducing legume intake. However, it may increase total forage intake. The aim of this study was to investigate how mixed stubble heights under rotational stocking affects grazing behavior, forage intake, and nitrogen balance. The trial was conducted at the Universidade Federal de Lavras, MG, Brazil for two consecutive years. The area was divided into 12 experimental units (paddocks) with three defoliation intensities (severe, moderate and light). The experimental area was constituted by marandu palisade grass (*Urochloa brizantha* cv. Marandu) and pintoi peanut (*Arachis pintoi* cv. Mandobi). The occupation period of the rotational mob stocking started when the canopy reached 24 to 27 cm and lasted for 9 days. It was used two Tabapua heifers per treatment as testers and put-and-take animals were added when it was necessary to achieve the planned stubble height for each treatment. Canopy characteristics, grazing behavior, instantaneous intake rate, nutritive value, intake, and N balance were evaluated in the first and last occupation day. Defoliation intensity (DI), season (S) and the interaction between the two (DI×S) were considered fixed effects, year and replicate were considered random effects. Light defoliation showed greater forage allowance and lower stocking rate than the other intensities (6.3 kg DM/kg LW and 1.3 AU/ha). Grazing time did not differ in the first (390 min; P=0.150) or the last occupation day (440 min; P=0.203). During the last day, 20 cm showed 70% greater intake rate (33.2 g DM/min) than the average of the other treatments, related to greater biting rate (38.0 bites/min) and bite weight (0.9 g DM/bite). Grass and total dry matter intake (DMI; %LW) were greater for moderate and light defoliations, but legume intake was lower for severe and moderate defoliation. The efficiency of microbial synthesis (EMS) was 101 g of microbial protein/kg DOM greater during summer compared to spring but did not vary according to DI (P=795). Stubble height of 15 cm increases SR without reducing DMI.

Keywords: *Arachis pintoi*, nitrogen balance, forage intake, nutritive value, *Urochloa brizantha*

1. INTRODUCTION

In order to maximize intake and performance for livestock production, the balance between quantity and quality of forage produced, which is also determined by canopy structure, must be achieved. In short duration grazing systems this balance may be succeeded by controlling the frequency and the intensity of grazing events (Motazedian and Sharrow, 1990a). Defoliation basically consists of removing part of the shoot organs and it is characterized by intensity and frequency (Gastal and Lemaire, 2015). Some recent studies suggest that more frequent (Gomes et al., 2018) and less severe grazing (Ruggieri et al., 2020) favor nutritive value, canopy structure and as a result, forage intake. Intake is the most important determinant of animal performance, and it can be influenced by non-nutritional characteristics, such as canopy structure and forage allowance (Poppi et al., 1999; Gomes et al., 2018; Ruggieri et al., 2020).

Forage quality comprehends not only the nutritive value of a forage, but also voluntary intake and the effects of any anti-nutritional factor that might be present (Collins et al., 2003). Nutritive value can decline as defoliation frequency is reduced, such as decreasing in crude protein concentration and digestibility, besides, lower grazing intensities contributes for a higher protein concentration on the forage consumed due to the greater opportunity of selection (Motazedian and Sharrow, 1990b; Newman et al., 2021). Defoliation intensity and frequency also affect the proportion of legume in the canopy (Tamele et al., 2018; Homem et al., 2019), reflecting on the nutritive value of the diet, once the nutritive value of legumes tends to be greater than for tropical grasses.

The nutritive value of diet influences the composition of materials returning as feces and urine. As an example, there is more excretion of nitrogen in the urine of animals fed with diets containing greater concentration of crude protein. However, despite the increasing on nitrogen excretion in urine, there is an increase in the nitrogen retained in the animals’ body (Gomes et al., 2020; Homem et al., 2021b; Homem et al., 2021a). Thus, frequency and intensity of grazing could affect, not only animal behavior and intake, as the composition of materials returning to the soil and its use by the body.

Thomas (1992) establishes an optimal range between 20 and 45% of legume proportion in a mixed pasture so the legume benefits to the system are maximized. Tamele et al. (2018) showed that mixed pastures of marandu palisade grass and pintoi peanut maintained between 30 and 40 cm had lower stolon density and less than 20% of legume in the forage mass, while pastures managed at 10 cm showed up to 60% of legume, but there is no data in the literature for intermittent stocking. The proper management of mixed pastures in an intermittent stocking system is required in order to maximize gains per area, as well as maintaining an adequate proportion of legume in the mixture exploiting the benefits of legume inclusion on the diet and the pasture, returning nitrogen to the system through excreta and litter.

Defoliation management has great effect on the canopy structure, behavior, intake, and nitrogen balance. Thus, we hypothesized that light defoliation would favor animal intake, due to the canopy structure and the greater opportunity of selection. The objective of this study was to determine a defoliation intensity that would favor forage intake with more quality, increasing the apparent efficiency of nitrogen utilization in the animal’s body.

2. MATERIAL AND METHODS

All experimental procedures were approved by the Ethics and Animal Welfare Committee of the Federal University of Lavras (protocol number 026/2019).

a. Experimental site

The study was conducted at the Experimental Farm of the Federal University of Lavras, Brazil (21°14′S, 45°00′W; 918 m above sea level). The area presents a subtropical humid mesothermal climate with dry winters (Köppen-Geiger climate classification: Cwa; (de Sá Júnior et al., 2012). Meteorological data were obtained from a weather station located 1,000 m from the experimental area (Fig. 1).

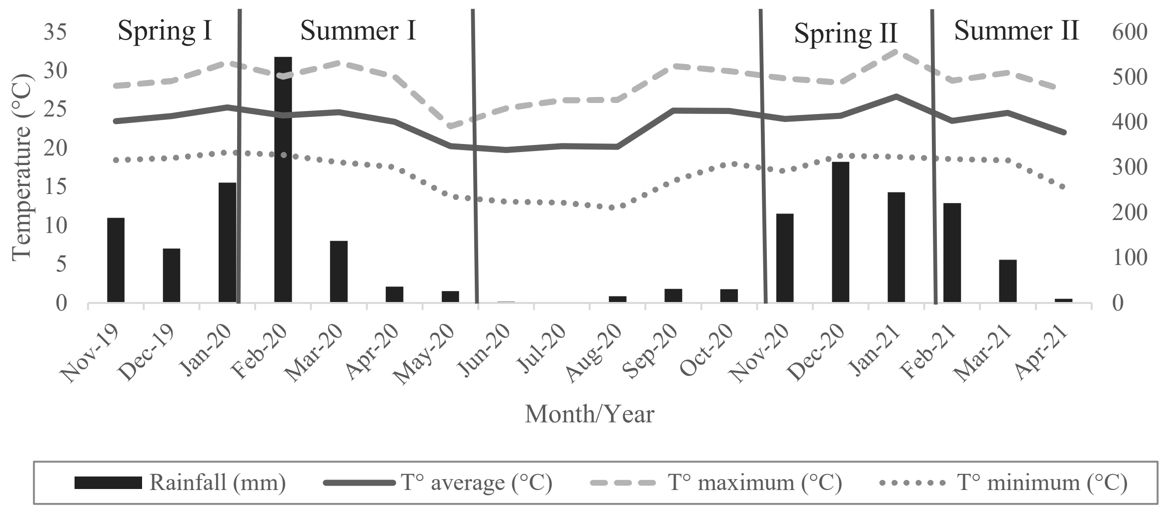
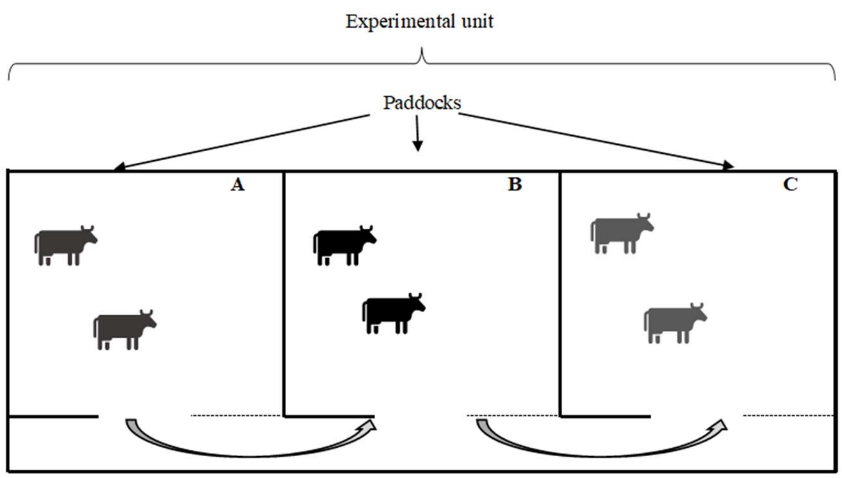


Figure 1. Monthly temperatures (°C) and rainfall (mm) in Lavras, Brazil, during the experimental period (seasons of the year).

The pasture used was established in December 2006 by joint seeding of marandu palisade grass (*Urochloa brizantha* syn. *Brachiaria brizantha* cv. Marandu) and pintoi peanut (*Arachis pintoi* cv. BRS Mandobi). The seeding rates were 7.6 and 7.2 kg/ha of pure live seed for palisade grass and pintoi peanut, respectively.

The soil of the experimental site at the start of the study (November 2019) were pH = 5.6, OM = 4.58 dag/kg, P = 2.82 mg/dm^3, P-rem= 24.8 mg/l, K^+ = 69.41 mg/dm^3, Ca^{2+} = 1.91 cmolc/dm^3, Mg^{2+} = 0.52 cmolc/dm^3, H + Al = 3.1 cmolc/dm^3, Al^{3+} = 0.09 cmolc/dm^3, and cation exchange capacity = 5.71 cmolc/dm^3. Fertilizers were applied as single superphosphate, potassium chloride. Applications (kg/ha) corresponded to 40 of P_2O_5, 53 of K_2O, according to the soil analysis. Thirty days before starting the experiment, it was also applied agricultural gypsum (1.39 t/ha) and limestone (278 kg/ha).

The pasture was divided into 12 experimental units (paddocks) and each paddock was subdivided into three paddocks of the same size (A, B, and C). The area for each treatment considered an occupation period of nine days by two animals weighing an average of 250 kg and consuming 2% of live weight (Fig. 2). Thus, each replicate of light defoliation had 1080 m^2, moderate had 660 m^2, and intense had 480 m^2. A 2-yr experimental period (from 2019 to 2021) was adopted, from November to January (Spring) and from January to April (Summer; Fig. 1). Assessments were not performed from May to September for both years due to the climatic conditions (dry winter).



Item	Period of ocupation (d)								
	1	2	3	4	5	6	7	8	9
Marker dosage	x	x	x	x	x	x	x	x	x
Fecal sampling							x	x	x
Urine sampling							x	x	x
Hand-plucked sampling							x		x
Forage sampling	x		x						
Animal behavior observation				x		x			
Intake rate evaluation							x		x
Sward structure evaluation	x								

Figure 2. The experimental unit was divided into three paddocks with 3-d occupation period in each one. A reserve mixed pasture of palisade grass and pintoi peanut was used to keep animals during rest periods. All the evaluations were performed as explained in the figure. Grazing behavior were assessed when no other evaluation was being performed to not interfere on the animals' natural behavior.

b. Treatments and experimental management

The grazing managements strategies consisted of three defoliation intensities (DI): severe (10 cm), moderate (15 cm), and light (20 cm of stubble height). The treatments were disposed on a completely randomized design with four replicates, equaling 12 experimental units. The rest period length was determined as a function of each treatment. Therefore, for the severe defoliation it was necessary a longer rest period. All paddocks were grazed when the canopy reached between 24 to 27 cm of height (Gomes et al., 2018). A minimum of two Tabapua heifers (265 ± 20 kg) were used to graze paddocks to the target stubble height for each treatment. The canopy height was assessed daily in the morning and additional put-and-take heifers were added to make the canopy achieve the treatment height during a 9-d period. Put-and-takes stayed for at least 24h in the paddock in order to account for stocking rate. Stocking density and stocking rate (animals/ha) were calculated dividing the number of animal units (AU; bovine weighing 500 kg) by paddock area and the number of AU by the estimated total area used to complete a grazing cycle, respectively (Allen et al., 2011).

c. Experimental evaluations

Canopy structure

The average canopy height (CH) was measured using a canopy stick (Barthram, 1985) at 80 random points for each paddock before and after grazing. The forage mass was evaluated in four strata of the CH (% of CH). The first stratum, closer to the ground, represented 0% to 25%, the second stratum 25% to 50%, the third stratum 50% to 75%, and the fourth stratum (at the top of the canopy) 75% to 100% of the CH (Tamele et al., 2018). The canopy structure was evaluated using an inclined point quadrat with 100 data points per experimental unit (Wilson, 1960). To achieve the forage mass of each stratum (% of mass per stratum), the frequency of each botanical component was multiplied by the respective mass of each botanical and morphological component in the four-canopy stratum. Forage mass was sampled by using three square frames of 0.5 × 0.5 m (pre- and post-grazing) per experimental unit, once for each grazing cycle (Fig. 2). After harvesting the forage, botanical and morphological compositions were assessed. The grass samples were separated into stem (stem + sheath), leaf (leaf blade), and dead material. Legume samples were separated into stem and leaf (stipule + petiole + leaflet). Forage samples were oven-dried at 55 °C for 72 h to a constant weight. Grass mass (kg/ha) was considered whole-plant without dead material. Legume mass (kg/ha) was considered the sum of leaf and steam masses. Total forage mass (kg/ha) was the sum of grass and legume components in the above ground canopy.

Ingestive behavior and short-term herbage intake

The daily grazing time (minutes) was assessed on two tester animals on each treatment. Data were collected from 6:00 am to 6:00 pm during the first and the third day of occupation on the second paddock after supplying titanium dioxide (Fig. 2). Each day, the heifers were followed individually by a trained observer from outside the paddock. Grazing activity was recorded each five minutes and we considered that the animal kept doing the activity until the next observation. Grazing events are interspersed with resting, ruminating and social activities (Bailey et al., 1996; Allen et al., 2011), so it was considered a new grazing event when the animal stopped grazing for more than 15 minutes.

The short-term herbage intake rate was determined by two rumen canulated cows in the first and third days of occupation for each treatment (Fig. 2) (McMeniman, 1997). The animals were kept in the corral overnight fasting. Before this assessment, all ruminal content was removed and kept in closed buckets, the rumen was dried with sponges, and it was assured by double check that the rumen was completely empty. Afterward, the animals were led to graze the paddock for five minutes tracked using a stopwatch and each bite was recorded with a manual counter by a trained observer, one for each animal. Then, the animals were taken to the chute to remove all forage consumed during grazing. All the samples were identified and put on plastic bags to be oven-dried 55 °C for 72 h until constant weight. After drying, the samples were weighted, and it was calculated the amount of forage ingested per minute (rate of intake; g DM/min) and the size of each bite (g DM/bite). The rate of bites was estimated by dividing the number of bites by the time observed (bites/min) (Chacon et al., 1976).

The recovery rate for the forage was assessed in 2019 and 2020 before the experimental evaluations started, aiming to check if this procedure was a good approach to evaluate short-term herbage intake. For this initial assessment, we took four rumen canulated cows and completely emptied their rumen, then the animals were allowed to consume 500 g/NM of palisade grass. When it was noticed that the animals had eaten all the palisade grass, the rumen was emptied again, and the sample was dried to estimate how much the forage was recovered. Samples of palisade grass were also dried to correct the equation based on dry matter ($y = 0.849x + 5.2134$; $R^2=0.9262$).

Nutritive value and herbage intake

Hand-plucked forage samples were collected for forage nutritive value analysis (de Vries, 1995). Samples from the first and the third day from each specie were evaluated separated (pre- and post-grazing). The samples were oven-dried at 55 °C for 72 h, and ground in a Cyclotec mill (Tecator, Herndon, VA) to pass a 1-mm screen. The DM of each sample was obtained by oven drying at 105 °C for 16h (INCT-CA G-003/1; Detmann et al., 2012). Ash concentration was determined by 4-h incineration process in a 600 °C muffle furnace (method INCT-CA M-001/1; Detmann et al., 2012). The crude protein (CP) concentration was calculated based on the N concentration (CP = total N × 6.25), which was determined using the Kjeldahl procedure (method INCT-CA N-001/1; Detmann et al., 2012). The neutral detergent fiber (NDF) was determined by the autoclave method at 105°C for 60 min (method INCT-CA F-002/1; Detmann et al., 2012). The ash and protein-free NDF was obtained by using sodium sulfite and incineration (Robertson, 1981).

Spot fecal samples were collected once a day varying the time of collection for each day (6:00 am, 12:00 pm, and 6:00 pm) and a composite sample was performed for each animal for the 3-d occupation period (Fig. 2). During sampling days, the heifers were brought from the paddocks to the chute to collect feces directly from the rectum. Titanium dioxide was used as external marker to estimate fecal production (Titgemeyer et al., 2001). The titanium was provided daily in the amount of 10 g/animal/d during nine consecutive days, six for adaptation, and three for collection. Fecal samples were oven-dried at 55 °C for 72 h to determine DM concentration, and air equilibrated, and ground in a Cyclotec mill (Tecator) to pass a 1-mm screen. The fecal samples were analyzed for titanium dioxide concentration according to Myers et al. (2004).

The proportion of palisade grass and pintoi peanut consumed was calculated based on the proportion of each component in the forage mass (Homem et al., 2021). The nutrient concentration in the diet was calculated by nutrient intake divided by the forage intake. The intake of OM, CP, and NDF (% BW/d) were also estimated.

Microbial protein synthesis

Microbial N synthesis (g N/d) was estimated by using the technique of purine derivatives in urine (Chen and Gomes, 1992). Spot sampling was used to assess the excretion of urinary nitrogenous compounds (Valadares et al., 1999). Spot urine samples were obtained by vulval stimulation at the same time as fecal sample collection. A 16 mL aliquot was taken and 4 mL of 0.002% sulfuric acid (H_2SO_4) was added. A 3-d composite sample was created and stored in a plastic flask at −20 °C. Urine creatinine concentration was determined using a commercial kit (Creatinine K016, Bioclin, Belo Horizonte, Brazil). Urine volume was estimated using creatinine concentration as a marker and assuming a daily creatinine output according to Eq. 1 (Silva et al., 2012).

$$UV\ (L/d) = (0.0345 \times BW^{0.9491}) \div UC_C$$

(Eq. 1)

where UV (L/d) is daily total urinary production, BW (kg) is body weight, and UC_C (g/L) is urine creatinine concentration.

Allantoin was analyzed as described by Chen and Gomes (1992). Uric acid was determined using a commercial kit (uric acid monoreagent, Bioclin). Excretion of allantoin and uric acid were estimated by multiplying their concentrations in urine by the daily urinary volume. Excretion of the purine derivatives in urine was calculated by the sum of the allantoin and uric acid excretions (mmol/day). The daily purine absorption (P_a) and the production of ruminal microbial N (g/d) were calculated using the equations, respectively (Chen and Gomes, 1992)

$$Pa = (PD_e - 0.385 \times BW^{0.75}) \div 0.85$$

$$NMIC = 0.727 \times P_a$$

Eq. 2 and 3

where P_a (mmol/day) is purine absorbed, PD_e (mmol/d) is purine derivatives excreted (uric acid and allantoin), BW (kg) is body weight, and NMIC (g N/d) is ruminal production of microbial nitrogen. The efficiency of microbial synthesis in the rumen (g microbial N/kg of digestible OM) was calculated by dividing the production of ruminal microbial N by the digestible OM intake (kg/day). The ratio of CP/digestible OM was calculated based on the intake of digestible OM and CP (g/kg). The ratio microbial protein/CP intake was calculated.

Nitrogen balance

The nitrogen balance was obtained by subtracting the total excreted N in the faces and urine of the total N intake, representing the total N that was effectively retained by the animal. For this purpose, the diet components, fecal, and urine samples were analyzed for N concentration, according to method INCT-CA N-001/1 (Detmann et al., 2012). In order to determine the fecal N excretion, the fecal production was multiplied by the total N concentration in the feces. Urinary N excretion was calculated using urinary volume and total N concentration in these samples. The N intake was obtained based on the value of forage intake by the animals and the total N concentration found in the hand-plucking. The apparent efficiency of N utilization (ENU) by the animal was calculated dividing the nitrogen balance/nitrogen intake.

d. Statistical analysis

The experimental design was entirely randomized with three treatments (defoliation intensities), four replications, and repeated measurements over time (seasons of the year). Data were analyzed using the mixed model method (Littell et al., 2000), performed by the MIXED procedure of SAS (SAS Institute, Cary NC). First and last days of occupation were evaluated individually and were not evaluated in the model. The effects of defoliation intensity and season were considered fixed effects, and year and replicate as random effects. The Akaike information criterion was used to choose the best (co)variance structure (Akaike, 1974). All variance components were estimated using the restricted maximum likelihood method. The treatment averages were estimated using the LSMEANS statement and compared using Student's t-test with $P \leq 0.10$. The statistical model for data analysis was as follows:

$$Yijkz = \mu + DIi + Yj + Sk + (GI \times S)ik + \varepsilon ijk$$

where Yijkz = value observed in the ith DI of the kth season of the jth year on the zth day; μ = overall average; DIi = fixed effect associated with ith defoliation intensity, i = 1, 2, 3; Yj = random effect associated with jth year, j = 1, 2; Sk = fixed effect associated with kth season, k = 1, 2; (DI × S)ik = fixed effect of interaction of ith DI with the kth season and the zth day. εijk = random error associated with the ith DI, the kth season, the jth year.

To evaluate the long-term effect of the different defoliation intensities on the canopy structure, the comparison between first and last grazing cycle were included in the model for the composition of canopy stratum. Their interactions were also considered.

3. RESULTS

a. Stocking rate and density

Stubble height (SH), stocking rate (SR), and stocking density (SD) are reported in Table 1. The average of canopy height was close to what was previously planned for each defoliation intensity. The percentage removal (%) was 56, 41.3 and 23.3 of canopy height for severe, moderate and light defoliations. Stocking rate and stocking density were affected by DI, S and the interaction DI×S. Stocking rate and density had increases of 2.8 and 26.8 AU/ha when defoliation was increased from light to severe, and 1.7 and 15.8 AU/ha from moderate to severe. During summer, the pasture supported 0.7 more AU/ha then during spring. Severe defoliation presented higher stocking rate (+1.1 AU/ha) and stoking density (+10.4 AU/ha) during summer compared to spring. The other intensities did not differ between the seasons (P>0.10). Forage allowance (FA; kg DM/ kg LW) presented opposite pattern as SR and SD, showing lower FA for severe, followed by moderate and light. Light defoliation presented 4.6 kg DM/kg LW greater FA than severe defoliation.

Table 1. Post-grazing and stocking characteristics of marandu palisade grass-pintoi peanut pastures influenced by defoliation intensity and season of the year

item	Defoliation intensity (DI)			SEM	Season (S)		SEM	*P-value*		
	severe	moderate	light		spring	summer		DI	S	DI*S
Canopy height (post-grazing)	10.6	14.9	19.9	0.5	.	.	.	.	.	.
Removal (%)	56.0	41.3	23.3	0.02	.	.	.	.	.	.
Stocking rate (AU/ha)							0.3	<0.01	<0.01	0.08
Spring	3.6 Ba	2.2 Ab	1.2 Ac							
Summer	4.7 Aa	2.5 Ab	1.4 Ac							
Stocking density (AU/ha)							2.7	<0.01	<0.01	<0.01
Spring	32.1 Ba	25.4 Ab	10.8 Ac							
Summer	42.5 Aa	22.9 Ab	10.9 Ac							
Forage allowance (kg DM/kg LW)	1.69 c	2.96 b	6.30 a	0.27	3.98	3.31	0.28	<0.01	0.17	0.73

a-c Least squares means within a row with different lowercase differ (P ≤ 0.10).
A-B Least squares means within a column differing uppercase differ (P ≤ 0.10).
SEM = standard error of the means
Abbreviations: DI = defoliation intensity. Severe = stubble height of 10 cm; moderate = stubble height of 15 cm; light = stubble height of 20 cm
S = seasons. Spring (from October to January) and Summer (from January to April)

b. Canopy structure

Considering only the first and the last seasons in the model, the interaction between DI, grazing cycle (GC) and strata (ST) was significant for mass of grass and legume (Fig. 3A, B, and C). There was no effect of DI, GC, nor DI×GC (P>0.10). The interaction between DI×ST was significant for mass of grass and legume (P<0.01).

Severe defoliation did not show any change in the proportion of grass and legume for the canopy upper layer, however moderate defoliation showed greater proportion of grass mass in the upper layer compared to the first evaluation (75 – 100%; Figure 3B). There was a decrease in the mass of marandu palisade grass and pintoi peanut for light defoliation in the layer closer to the ground (0 - 25%; Fig. 3C). Light defoliation showed an increase in the proportion of grass and legume in the upper layer between the first and the last grazing cycle. In the last cycle, light defoliation showed greater legume mass on the two upper layers (50 – 75 and 75 – 100%) compared to the other defoliation intensities.

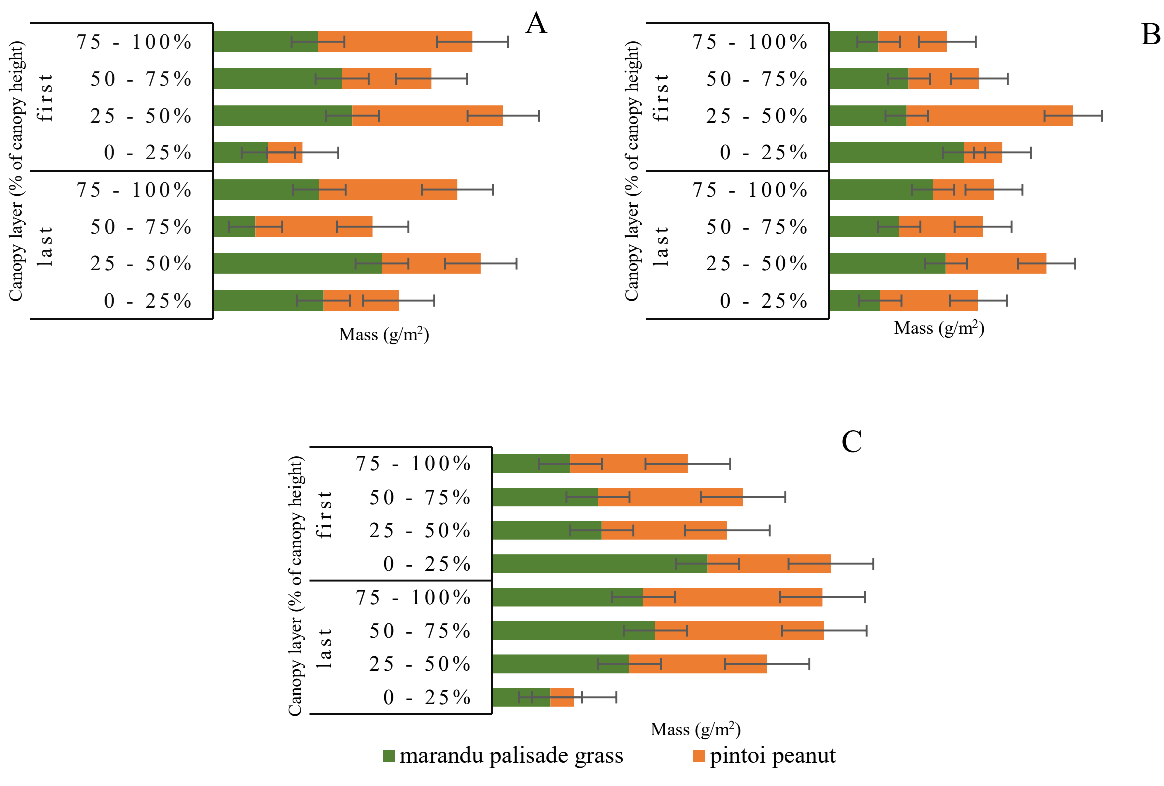
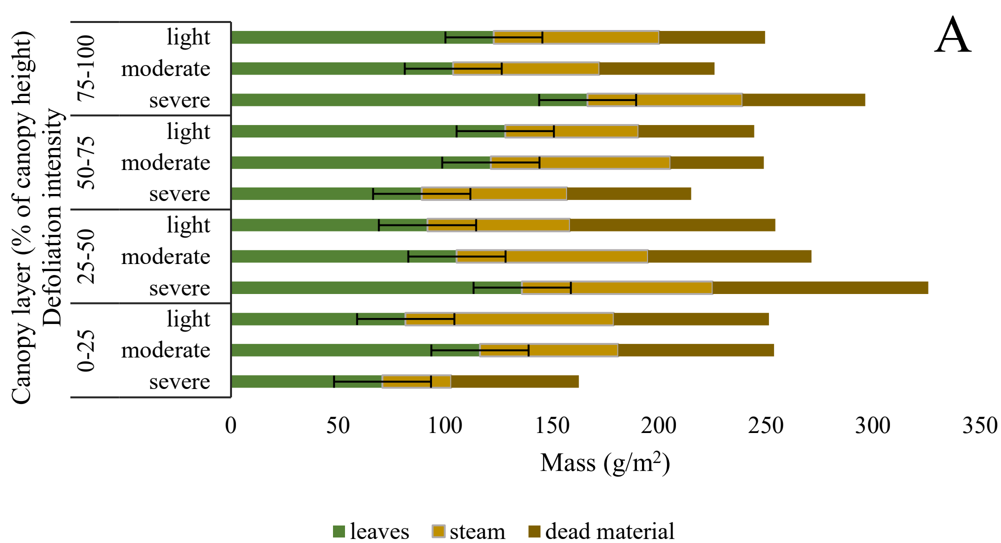


Figure 3. Pre-grazing vertical distribution of palisade grass and pintoi peanut according to defoliation intensity (A: severe; B: moderate; C: light), stratum, and grazing cycle (first: spring 2019; last: summer 2020). Error bars represent ± standard errors of the means

Considering all four seasons evaluated, there was an interaction between defoliation intensity and stratum for grass (P<0.01) and legume leaves (P=0.02). The interaction between stratum and DI for grass and legume leaves are shown in figure 4 A and B. Moderate defoliation showed a constancy of grass leaves mass among layers (Fig. 4; B), while light and severe defoliations showed an increase in leaves mass on the upper layer of the sward. Same pattern occurred with legume leaves (Fig. 4; B), which presented uniform among canopy layers for moderate defoliation while it increased on the upper layer for intense and light defoliations.



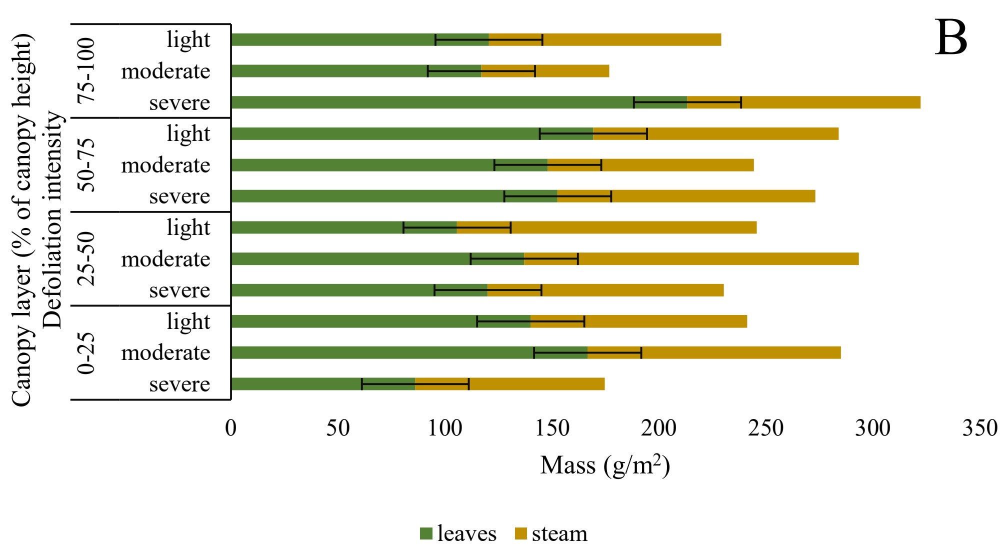


Figure 4. Pre-grazing vertical distribution of marandu palisade grass and pintoi peanut according to defoliation intensity and stratum (A: palisade grass; B: pintoi peanut). Error bars represent ± standard errors of the means

c. Ingestive behavior and short-term herbage intake

Data of short-term intake did not differ between defoliation intensities or season during the first day of occupation (Table 2). There was no interaction between defoliation intensities and season for any of the variables evaluated (P>0.10). The heifers spent about 44 more minutes grazing during summer comparing to spring. Considering the last day of occupation, biting rate (bites/min) for light defoliation was 59% greater comparing with the average of moderate and severe, it was visually observed that the canulated animals sometimes refused to graze paddocks with lower stubble heights. Bite weight (g DM/min) was lower for severe grazing comparing with the other intensities. Intake rate (g DM/min) was 116 and 65% greater for light defoliation compared to severe and moderate. There was no difference between intensities on minutes grazing (P>0.10). However, heifers grazing on severe and moderate intensities used to graze the aisle between paddocks searching for forage. There was an increase of 15 grazing events to severe grazing compared to light grazing, even though the minutes grazing did not differ.

Table 2. Ingestive behavior and short-term intake of heifers grazing palisade grass-pintoi peanut pastures influenced by defoliation intensity and season of the year

item	Defoliation intensity (DI)			SEM	Season (S)		SEM	P-value		
	severe	moderate	light		spring	summer		DI	S	DI*S
First-occupation-day ingestive behavior and short-term intake										
biting rate (bites/min)	46.2	40.9	45	2.8	43.6	44.5	2.4	0.32	0.77	0.52
bite weight (g DM/bite)	1.1	1.3	1.2	0.2	1.2	1.2	0.2	0.06	0.58	0.78
intake rate (g DM/min)	50.4	53.4	52.1	5.8	52.3	51.7	5.6	0.77	0.85	0.18
grazing time (min)	378	414	384	6.0	372	414	6.0	0.15	<0.01	0.63
grazing events	21.0	20.0	21.0	5.2	19.0	23.0	5.2	0.83	0.06	0.34
Last-occupation-day ingestive behavior and short-term intake										
biting rate (bite/min)	22.0 b	28.1 b	38.0 a	3.2	28.0	30.7	2.7	<0.01	0.43	0.51
bite weight (g DM/bite)	0.6 b	0.8 a	0.9 a	0.2	0.8	0.8	0.2	0.02	0.60	0.16
intake rate (g DM/min)	15.4 c	22.7 b	33.2 a	4.3	22.4	25.1	4.1	<0.01	0.43	0.33
grazing time (min)	432	456	432	6.0	438	444	6.0	0.20	0.44	0.34
grazing events	35.0 a	25.0 ab	20.0 b	10.2	25.0	30.0	10.2	0.05	0.22	0.57

a-c Least squares means within a row with different lowercase differ ($P \leq 0.10$).
SEM = standard error of the means
Abbreviations: DI = defoliation intensity. Severe = stubble height of 10 cm; moderate = stubble height of 15 cm; light = stubble height of 20 cm
S = seasons. Spring (from October to January) and Summer (from January to April)

d. Nutritive value and forage intake

Pre-grazing crude protein (CP), neutral detergent fiber (NDF), and organic matter (OM) for marandu palisade grass were unaffected by DI (Table 3). However, CP and OM presented an interaction between DI×S ($P<0.10$). Severe defoliation during summer presented an increase of 1.5 g/100 g DM on the CP concentration compared with summer, the other intensities did not change. For pintoi peanut there was only a slightly increase (0.5%) on OM for severe defoliation during summer, the other characteristics were not affected by none of the factors evaluated ($P > 0.10$).

Post-grazing crude protein concentration of marandu palisade grass was 1.4 g/100 g DM greater for light defoliation comparing to the other intensities, which did not differ from each other. OM and NDF were lower during spring compared to summer, but CP presented opposite pattern, which showed 1.3 g/100 g greater during this season. Regarding to post-grazing pintoi peanut, CP concentration was unaffected by DI, S or DI×S ($P>0.10$). NDF concentration was 6.6 g/100 g greater during summer compared to spring for moderate defoliation and 7.6 g/100 g greater for light. OM concentration showed an increase of 1.3 g/100 g during summer compared to spring.

Table 3. Pre- and post-grazing nutritive value of marandu palisade grass-pintoi peanut pastures influenced by defoliation intensity and season of the year

item	Defoliation intensity (DI)			SEM	Season (S)		SEM	P-value		
	severe	moderate	light		spring	summer		DI	S	DI×S
First-occupation-day nutritive value of marandu palisade grass and pintoi peanut (g/ 100 g DM)										
marandu palisade grass										
CP								0.21	0.03	0.10
spring	10.2 Aa	8.9 Ab	9.2 Aab	0.3						
summer	8.7 Ba	8.3 Aa	9.3 Aa							
NDF	67.9	67.1	69.4	1.5	68.1	68.2	1.3	0.38	0.89	0.74
OM								0.12	<0.01	<0.01
spring	92.1 Aa	92.1 Aa	92.3 Aa	0.1						
summer	91.6 Aa	92.1 Aa	91.0 Bb							
pintoi peanut										
CP	17.0	18.1	17.7	0.4	17.9	17.4	0.4	0.19	0.39	0.57
NDF	54.5	52.8	55.5	2.4	54.5	54.3	2.3	0.42	0.88	0.23
OM								0.91	0.18	0.08
spring	91.9 Ba	92.2 Aa	92.2 Aa	0.7						
summer	92.7 Aa	92.2 Aa	92.2 Aa							
Last-occupation-day nutritive value of marandu palisade grass and pintoi peanut (g/ 100 g DM)										
palisade grass										
CP	6.4 b	6.0 b	7.6 a	0.4	7.3	6	0.4	<0.01	0.01	0.28
NDF	75.4	75.3	72.5	1.9	71.9	76.8	1.7	0.40	0.02	0.28
OM	90.6 b	91.1 ab	92.1 a	0.5	90.7	91.9	0.5	0.03	0.07	0.65
pintoi peanut										
CP	12.8	13.3	14.5	0.7	13.8	13.3	0.9	0.20	0.45	0.25
NDF								0.02	0.02	0.03
spring	60.1 Aa	54.0 Bb	58.2 Bab	7.9						
summer	57.6 Ab	60.6 Ab	65.8 Aa							
OM	91.3 b	91.6 ab	91.8 a	0.3	90.9	92.2	0.3	0.04	<0.01	0.55

a-c Least square means within a row with different lowercase differ (P ≤ 0.10).
A, B Least square means within a column differing uppercase differ (P ≤ 0.10).
SEM = standard error of means
Abbreviations: DI = defoliation intensity. Severe = stubble height of 10 cm; moderate = stubble height of 15 cm; light = stubble height of 20 cm
S = seasons. Spring (from October to January) and Summer (from January to April). CP = crude protein; NDF = ash and protein-free neutral detergent fiber; OM = organic matter

Intake and apparent digestibility of forage consumed is presented in Table 4. Grass, legume, total dry matter (DMI), organic matter (OMI), crude protein (CPI), and ash and protein-free neutral detergent fiber (NDFI) intakes (% LW) were affected by DI (P<0.10). Only grass intake (% LW) was influenced by season, which was 0.2% greater during spring than during summer. Moderate defoliation showed similar grass intake to light (1.3 %LW), however legume intake and CPI were lower for moderate and did not differ from severe (1.25 and 0.29% LW in average). For moderate and light defoliations, DMI and OMI were in average 2.7 and 2.5 %LW, greater than for severe defoliation. Spring caused an increase in the apparent digestibility of DM and CP (P<0.10), but not for OM or NDF (P>0.10). DM, OM, CP, and NDF apparent digestibilities were not affected by DI or DI×S. The average for the digestibilities were 67.3% (DM), 71.6 % (OM), 72.6% (CP), and 70.0% (NDF).

Table 4. Diet nutritive value, intake and apparent digestibility of forage consumed by beef heifers in marandu palisade grass and pintoi peanut as affected by defoliation intensity and season of the year

item	Defoliation intensity (DI)			SEM	Season (S)		SEM	P-value		
	severe	moderate	light		spring	summer		DI	S	DI×S
Diet nutritive value (g/ 100 g DM)										
OM	85.7	91.8	91.8	3.5	91.4	88.2	2.8	0.37	0.44	0.43
CP	11.2 b	11.4 b	12.7 a	0.5	12.2	11.3	0.4	0.10	0.23	0.11
NDF	64.5	67.9	69.1	5.1	67.5	66.9	4.9	0.45	0.81	0.52
Intake, %LW										
Grass	1.1 b	1.3 a	1.3 a	0.10	1.30	1.10	0.08	0.07	0.02	0.53
Legume	1.2 b	1.3 b	1.5 a	0.10	1.30	1.30	0.08	0.04	0.99	0.57
Total DM	2.2 b	2.6 a	2.8 a	0.16	2.7	2.4	0.14	0.02	0.12	0.84
OM	2.1 b	2.4 a	2.6 a	0.13	2.5	2.2	0.12	0.02	0.24	0.71
CP	0.28 b	0.30 b	0.35 a	0.02	0.33	0.29	0.02	0.01	0.21	0.71
NDF	1.6 c	1.8 b	1.9 a	0.09	1.80	1.70	0.10	<0.01	0.50	0.67
Apparent digestibility, %										
DM	64.8	67.5	69.7	2.4	69.4	65.2	2.2	0.23	0.07	0.97
OM	71.0	70.8	72.9	0.9	72.7	70.4	0.9	0.17	0.12	0.58
CP	72.8	71.0	74.1	1.3	78.1	71.1	1.1	0.26	0.10	0.75
NDF	68.6	69.6	71.7	3.0	70.3	69.6	3.0	0.10	0.65	0.56

a-c Least squares means within a row with different lowercase differ (P ≤ 0.10).
A, B Least square means within a column differing uppercase differ (P ≤ 0.10).
SEM = standard error of means
Abbreviations: DI = defoliation intensity. Severe = stubble height of 10 cm; moderate = stubble height of 15 cm; light = stubble height of 20 cm
S = seasons. Spring (from October to January) and Summer (from January to April).
CP = crude protein; NDF = ash and protein-free neutral detergent fiber; OM = organic matter, %LW = percentage of live weight.

e. Nitrogen balance

Nitrogen excretion in urine (UNE, g/d/heifer) and total nitrogen excretion (TNE, g/d/heifer) were not affected by DI (P>0.10). However, were greater during spring than summer (Table 5). Nitrogen excreted in the feces (FNE, g/d/heifer) showed an interaction between DI×S (P = 0.065). During spring, moderate and light defoliation were in average 11.3 g/d/heifer greater than for severe defoliation, this difference did not remain during Summer, when there was no difference between severe and moderate defoliation. TNE (g/d/heifer) presented an interaction between DI×S (P = 0.068), which did not was affected by season for severe but was greater during spring for moderate and light defoliations. When it is considered the FNE, UNE and TNE per hectare during the whole season, there was an increase for severe defoliation, so even though TNE per animal was smaller for severe defoliation, the higher stocking rate led to a greater N excretion per hectare. Apparent efficiency of nitrogen utilization (EUN, g/g) was only influenced by S (P = 0.007), showing a difference of 0.36 g/g between spring and summer. Besides the difference of 8.6 g of N between light and severe defoliation for N balance, there was no statistical differences between DI or DI×S (P>0.10), but there was an increase of 28.3 g N during summer compared to spring. Crude protein/digestible organic matter ratio (CP/DOM, g/kg) showed an interaction between DI×S (P=0.08), which light defoliation presented 31 g/g greater CP/DOM during the summer compared to spring. Microbial synthesis (MP, g of protein/day) was not affected by DI, S, and DI×S (P>0.10). The efficiency of microbial protein synthesis (EMS, g of microbial protein/kg DOM) was 101 g MP/g DOM greater during summer than during spring but did not change between defoliation intensities.

Table 5. Nitrogen metabolism by beef heifers in palisade grass and pintoi peanut as affected by defoliation intensity and season of the year

item	Defoliation intensity (DI)			SEM	Season (S)		SEM	P-value		
	severe	moderate	light		spring	summer		DI	S	DI×S
UNE, g/d/heifer	26.2	28.9	30.5	6.0	39.5	17.6	4.6	0.85	<0.01	0.39
FNE, g/d/heifer							4.7	<0.01	0.07	0.07
spring	56.5 Bb	68.8 Aa	66.7 Aa							
summer	66.2 Ab	65.5 Ab	73.2 Aa							
TNE; g/d/heifer							9.3	0.27	<0.01	0.07
spring	90.1 Ab	111.4 Aa	108.9 Aa							
summer	85.0 Aa	80.7 Ba	92.1 Ba							
EUN, g/g	0.110	0.220	0.220	0.100	0.002	0.370	0.100	0.49	0.01	0.54
N retention, g N	18.0	18.2	26.6	5.0	6.8	35.1	5.5	0.35	<0.01	0.61
CP/DOM, g/kg							11.8	0.33	0.49	0.08
spring	211.5 Aa	183.2 Aa	187.2 Ba							
summer	192.2 Aab	189.8 Ab	218.2 Aa							
MP, g of protein/day	273.2	272.4	188.8	70.3	228.3	261.3	55.1	0.52	0.45	0.95
EMS, g of microbial protein/kg DOM	209.3	167.1	179.1	47.1	134.5	235.8	36.3	0.79	0.03	0.48

a-c Least square means within a row with different lowercase differ (P ≤ 0.10).

A, B Least square means within a column differing uppercase differ (P ≤ 0.10).

Abbreviations: DI = defoliation intensity. Severe = stubble height of 10 cm; moderate = stubble height of 15 cm; light = stubble height of 20 cm

S = seasons. Spring (from October to January) and Summer (from January to April).

CP/DOM, crude protein/digestible organic matter ratio; ENU, apparent efficiency of nitrogen utilization in the animals' body; FNE, feces nitrogen excretion; MP, microbial synthesis; UNE, urinary nitrogen excretion; TNE, total nitrogen excretion; EMS, efficiency of microbial protein synthesis; SEM, standard error of the means.

4. DISCUSSION

Stocking rate, defined by Allen et al. (2011) as the relation between the total number of animals and the total area utilized for grazing in a specific period, is one of the most important management variables that could define the animal and vegetal productivity of a grazing system (Venter et al., 2021). However, stocking rate should not be considered disconnected from other factors as forage intake and its chemical composition, since all these factors can influence animal performance. The ideal grazing management should maximize quantity as much as quality of forage available, in a way to improve individual performance and gain per area, which it is not always possible to achieve (Jones and Sandland, 1974; Sollenberger and Cherney, 1995). There was greater stocking rate and stocking density during summer compared to spring (Table 1), related to better environmental condition, as temperature, humidity, and rainfall, and consequently, higher photosynthetic rates and herbage accumulation during this season. Greater stocking rates during summer were found by Sbrissia et al. (2020) and Homem et al. (2021a), both studying tropical forages. Greater forage allowance for the light defoliation intensity was a result of the treatment imposed (Table 1). Forage allowance can provide a target for desired animal gains based on forage utilization and grazing intensities (Rouquette, 2016). Silva et al. (2019) reported an increase of 12.4 kg DM/kg LW when the residual leaf area index (rLAI) was reduced from 2.6 to 0.8, and consequently the authors reported a linear increase in the average daily gain as the rLAI increases.

In order to observe the changes caused in the canopy along the 2-year experimental period, it was compared the first grazing cycle to the last grazing cycle to each defoliation. There was an interaction between defoliation intensity, grazing cycle, and stratum for grass and legume mass. It was expected that taller canopies would cause an increase of legume mass on the upper layer, once the legume tends to grow in a vertical direction, a strategy used to expose itself to sunlight (Tamele et al., 2018; Cruz et al., 2020; Ferreira et al., 2021). Severe defoliation showed an increase in grass and legume mass in the base of the sward, while imposing light defoliation increased legume and grass mass on the two upper layers. During the first grazing cycle, the legume showed a contribution of around 65% of total mass for the three defoliation intensities in the upper layer (75 – 100%). However, for the last grazing cycle, the legume showed an increase of 77% for severe to 85% of total mass for light defoliation, which corroborates Tamele et al. (2018) and Ferreira et al. (2021).

There was an interaction between stratum and defoliation intensity when the grazing cycles were grouped into seasons for grass and legume leaves, but not for dead material and steams (Table 2). The distribution of components along the canopy stratum tends to be constant regardless of the forage species and growing habits (Fonseca et al., 2013; Silva et al., 2018; Tamele et al., 2018), which validate the results found on the study. Most of the leaves were concentrated in strata easily grazed by animals (above 50% of canopy height), and consequently, mass of leaves was influenced by defoliation intensity (Fig. 4 A and B). The canopies can adapt their structure to defoliation, particularly regarding to tiller size and density, leaf length, and number of leaves per tiller, mostly modulated by light quantity and quality (Gastal and Lemaire, 2015; Cruz et al., 2020). Only moderate defoliation showed constant mass of grass and legume leaves among the four strata. The constant distribution of leaves between canopy layers for moderate defoliation would facilitate forage intake in this defoliation intensity, once leaves are easier to prehend than other forage components.

Canopy structure is directly related to animal intake rate (McGilloway et al., 1999; Carrère et al., 2001; Ferreira et al., 2021). During the first day of occupation, the canopy structure did not differ between defoliation intensities, since all treatments began being grazed when the canopy reached 24 to 27 cm. However, as the canopy height decreases, leaf proportion decreases and steam and dead material increases, which makes harder for the animal to apprehend forage (Chacon and Stobbs, 1976; McGilloway et al., 1999). Thus, as upper layers are removed, there is a reduction on the opportunity of selection and the animals are forced to consume more steams and dead material, and to take smaller bite size in order to harvest the remaining leaf (Chacon and Stobbs, 1976). Some studies show that when the canopy height is reduced by defoliation, it tends to increase biting rate to compensate the smaller bite size (Chacon and Stobbs, 1976; Fonseca et al., 2013; Silva et al., 2018). However, contrasting with the literature (Chacon and Stobbs, 1976; McGilloway et al., 1999; Silva et al., 2018; Ferreira et al., 2021), in this study there was a decrease in biting rate caused by smaller canopies. It was observed that cows used to this evaluation spent more time searching for better plant components, as green leaves, and refused to graze lower canopies (10 and 15 cm), decreasing biting rate. It is important to emphasize that the highest stubble height had 20 cm which represented only 23% of canopy height removal, so for this treatment the canopy was mostly composed by leaves, making grazing easier and maintaining a greater intake rate, close to 37.2 g/min found by Ferreira et al., (2021) for 20% of removal of marandu palisade grass and pintoi peanut pastures.

There was no difference between moderate and light defoliations to total dry matter intake (DMI), both showed an intake of ± 1.7 %LW (Table 4). Severe defoliation modifies the canopy structure and compromise forage intake and performance, once there is a reduction in biting rate and biting size (Table 2). Among all the parameters of ingestive behavior, biting size is the most responsive to differences in the canopy conditions. As canopy height increases, as well as forage mass, bite size increases linearly in tropical and temperate canopies (Coleman et al., 1989). Hendricksen and Minson (1980) estimates that reducing bite size by 320 mg of OM over a 12-day period would decrease intake in 1 kg/d.

When the nutritive value was assessed in the first day of occupation, there was an increase in the CP of palisade grass during the summer, but not for pintoi peanut (Table 4). It is normal for forages to lose their nutritive value during drier seasons due to unfavored environmental conditions, but in the beginning of the rainy season (spring), there are greater growth rates and tissue renovation (Homem et al., 2021a) which could have led to greater crude protein concentration (CP) and crude protein and dry matter apparent digestibilities during this season (Table 3 and 4). Gomes et al. (2018) studied the effect of four grazing managements over the nutritive value of marandu palisade grass and pintoi peanut in the same area and reported greater CP concentrations than what was found in this study (9.9 and 18.7 vs. 9.1 and 17.3 g/100 g DM, respectively). Post-grazing, light defoliation showed greater CP concentration for marandu palisade grass than the average of the other intensities (7.6 vs. 6.2 g/ 100 g DM), mostly due to the greater proportion of grass and legume leaves for this intensity. Regarding to pintoi peanut, this forage showed greater concentrations of NDF for lower stubbles heights and during the summer. Pastures with lower stocking rates (SR) tend to allow better nutritive value than pastures under higher SR, due to greater selection opportunities, greater proportion of leaves and lesser steams and dead material in the upper stratum (Cook and Stoddart, 1953; Ruggieri et al., 2020) (Fig. 3 and 4).

There is variation in the chemical composition of forages due to maturity and season (Garcia et al., 2021). There is a canopy renovation after the dry season, with the growing of new tillers, which is composed by greater proportion of mesophyll cells that are tissues with greater digestibility. The greater CP apparent digestibility during the spring could have led to an increase in TNE (g/heifer/d) during this season, even though the legume intake did not vary (Table 6). UNE (g/d/heifer) was not influenced by DI and presented an average of 28.5 g/d, this value was similar to 29.5 g/d found by Gomes et al. (2018) for heifers grazing mixed pastures with 95% of LI and stubble height of 15 cm and within the range of 11.5 and 154 g/d reported by Waldrip et al. (2013) in a meta-analysis done to predict nitrogen excretion by beef cattle. FNE showed an average of 66.2 g/d/heifer and TNE was 94.7 g/d/heifer, both are in between the range of 35.1 to 80.7 g/d for FNE and 47.5 to 217 g/d described by Waldrip et al. (2013) for beef cattle. The deposition of total excreta per hectare and per season increases according to defoliation intensity even if the TNE (g/d/heifer) is lower for severe defoliation. Thus, there is an increase in TNE (kg/ha/season) when severe defoliation is imposed due to the greater stocking rate, while litter deposition appears to be evenly distributed regardless the stocking rate (Dubeux and Sollenberger, 2020).

ENU (g/g) was comparable to 0.23 g reported by Batista et al. (2017) to beef cattle fed with hay of Tifton 85 containing around 100 g/kg of CP and 720 g/kg of NDF. The rumen metabolism has been identified as the most important factor determining nitrogen efficiency (Calsamiglia et al., 2010). ENU is usually low in grazed forages because the ruminal degradation is inefficient and due to the low concentration of fermentable carbohydrates (Schwab and Broderick, 2017). Besides, cattle are more efficient when consuming a low protein diet, because their body takes advantage of N recycling (Calsamiglia et al., 2010; Schwab and Broderick, 2017). Our heifers were consuming a diet containing about 12.5% of CP, which could explain the low ENU.

Nitrogen retention, or balance is an indicative of N surplus or shortage to sustain MP synthesis in the rumen (Hristov et al., 2019). The three DI tested in this trial presented positive N balance (±20.9 g N), indicating a surplus of N that does not need to be compensated by a movement of N into the rumen to maintain fermentation (Hristov et al., 2019; Silva et al., 2019).

The CP:DOM ratio is used as an indicator of protein to energy status, Detmann et al. (2014) reported a maximum intake at 288 g CP/kg DOM. All defoliations intensities and seasons evaluated showed lower values of CP/DOM ratio, with light defoliation during the summer showing the greater value for this variable (Table 6). The voluntary intake is depressed when the ratio energy and protein is low due to ATP deficiency caused by the greater consumption by the urea cycle, increased heat production and animal indisposition (Detmann et al., 2014). All defoliation intensities showed more than 100 g/kg of CP:DOM that would not depress intake, however the low forage allowance observed for severe defoliation caused lower biting rate, bite mass and consequently decreased total DM intake (Table 5). During spring Gomes et al. (2018) reported 23% more condensed tannins in the forage peanut compared to summer, which could have increased the nitrogen retention and MP synthesis during this season. Condensed tannins inhibit protein degradation in the rumen and increases N recycling, it can be beneficial in order to take advantage of N recycling (Barry et al., 1986; Calsamiglia et al., 2010; Schwab and Broderick, 2017).

5. CONCLUSION

Despite the greater stocking rate under intense defoliation, there was a decrease in forage intake by animals grazing this defoliation intensity. Thus, moderate defoliation (15 cm of stubble height) showed greater stocking rate without affecting forage intake and nutritive value. Nitrogen is an important component for productivity of ruminants, but if it is excreted in excess can contribute to environmental contamination. Efficiency of nitrogen utilization was not affected by defoliation intensities, neither the nitrogen excreted in the urine. The three treatments showed positive nitrogen balance but did not differ from each other.

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GENERAL CONCLUSIONS AND IMPLICATONS

Mixing legume forages to pastures of tropical grasses can bring many benefits to the environment and to beef production system; however, it is necessary to maintain adequate proportion of the legume forage in the herbage mass by controlling the intensity and frequency of defoliation. Besides it is essential to correctly determine the diet nutritive value and intake in order to determine the best management or supplementation strategy. Hand-plucking is the best approach to determine the diet nutritive value in a mixed pasture of pintoi peanut and marandu palisade grass, due to the low cost and labor, and the capacity of incorporating the differences of animals' grazing selection. Despite the greater stocking rate under intense defoliation, there was a decrease in forage intake by animals grazing this defoliation intensity. Thus, moderate defoliation (15 cm of stubble height) showed satisfactory stocking rate, forage intake, and apparent efficiency of nitrogen utilization in the animal's body without changing the nutritive value of the diet.

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