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Defoliation patterns and tillering dynamics in Italian ryegrass under different herbage allowances

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ABSTRACT. This work was carried out in order to generate information on the tillering process and to contribute for the understanding of the defoliation process on Italian ryegrass (*Lolium multiflorum* Lam.) grazed by lambs under different herbage allowances: 6, 8 and 12 kg of dry matter per 100 kg of animal live weight per day. The grazing method was intermittent. The experimental design was completely randomized following a repeated measure arrangement, with three treatments and two area replications. The intensity of defoliation is greater at 6 and 9% herbage allowances. The defoliation interval is higher at 12% herbage allowance, intermediate at 9% and lower at 6%. The rates of tiller emergence, survival and death, stability index the tiller population are similar for the different herbage allowances and differ according to the phenological stage of ryegrass. Structural changes caused by canopy management with low herbage allowance and/or due to the advance in developmental stage of ryegrass alter defoliation strategies, with more intense and frequent grazing.

Keywords: accumulated thermal sum, intermittent grazing, defoliation intensity, defoliation interval, lambs, *Lolium multiflorum*.

Padrões de desfolhação e da dinâmica de perfilhamento em azevém sob diferentes ofertas de forragem

RESUMO. Este trabalho foi conduzido com o objetivo de gerar informações sobre o processo de perfilhamento e contribuir para com o entendimento do processo de desfolhação do azevém (*Lolium multiflorum* Lam.) utilizado por cordeiras, submetidas a diferentes ofertas de forragem: 6; 8 e 12 kg de MS 100^{-1} kg de peso corporal. O método de pastejo foi intermitente. O delineamento experimental foi inteiramente casualizado, com medidas repetidas no tempo, três tratamentos e duas repetições de área. A intensidade de desfolhação é superior nas ofertas de forragem 6 e 9%. O intervalo entre desfolhações foi superior na oferta de forragem 12%, intermediária na oferta 9% e inferior na oferta 6%. As taxas de aparecimento, sobrevivência e mortalidade de perfilhos e o índice de estabilidade da população de perfilhos são similares nas ofertas de forragem e variam nos estádios fenológicos do azevém. Mudanças estruturais do dossel causadas pelo manejo com baixas ofertas de forragem e/ou pelo avanço do estádio fenológico do azevém alteram as estratégias de desfolhação, com pastejo realizado de forma mais intensa e frequente.

Palavras-chave: soma térmica, pastejo intermitente, intensidade de desfolhação, intervalo entre desfolhações, cordeiras, *Lolium multiflorum*.

Introduction

In recent years, studies on forage crops have sought to relate plant response to management, based on criteria associated with abundant biomass, such as herbage allowance. This allowance plays an essential role in animal performance and in the productive response of pastures; depending on its value, it can influence the defoliation process by altering canopy structure. According to Lemaire et al. (2009), the amount of herbage removed results from the growth rate of new plant tissue and the efficiency of the harvesting process.

Defoliation patterns can be studied by evaluating the intensity and frequency of defoliation in individual tillers (SANTOS et al., 2013), and are essential to understanding the effect of grazing on plant and animal performance. Knowledge of the defoliation process allows management with greater harvesting opportunities and improved efficiency of produced herbage.

Pontes et al. (2004) observed in Italian ryegrass (*Lolium multiflorum* Lam.), under continuous grazing, that defoliation intensity decreases and the interval between defoliations increase as canopy heights get taller. For his part, Confortin et al.

(2010a), while evaluating the intensities of biomass removal from ryegrass intercropped with black oat, under intermittent grazing, observed that defoliation intensity was not influenced by the intensity of biomass removal. Machado et al. (2011) evaluated the effect of two intensities of biomass removal under intermittent grazing, and observed that defoliation intensity was similar whereas defoliation frequency was greater at the 'High' removal intensity (52% biomass removal). Understanding how differential herbage allowances affect the defoliation process of ryegrass can contribute to support higher yields and greater efficiency of use for this forage species.

One of the main characteristics of forage grasses, which guarantees their persistence after cutting or grazing, is the regeneration capacity of leaf tissue through leaf emergence from the remaining meristems or axillary shoots by tillering (ALEXANDRINO et al., 2004). Few studies have been carried out in Brazil to evaluate the tillering process in ryegrass (BARTH NETO et al., 2013).

The objective of the this work is to generate information on the tillering process of Italian ryegrass, as well as contribute to understanding the defoliation process under different herbage allowances.

Material and methods

The experiment was carried out at the Animal Science Department of the Federal University of Santa Maria (UFSM), located in the Central Depression region of Rio Grande do Sul state, Brazil, featuring soil classified as Paleaudalf. Weather data for the experimental period (Figure 1) were obtained from Inmet (2012).

The experimental area consisted of 0.6 hectare, divided into six plots, which represented the experimental units, plus an adjacent area of 0.4 hectare. The pasture of Italian ryegrass (*Lolium multiflorum* Lam.) cv. 'Comum' was implemented in May 2012, by broadcast seeding. At sowing time, 200 kg ha^{-1} of 05-20-20 (N-P-K) fertilizer were used. Urea was applied (100 kg of nitrogen per hectare), as topdressing, over four applications, on June 6, July 7, August 18, and September 15, 2012.

The intermittent grazing method was adopted, and three herbage allowances were evaluated: 6, 9 and 12 kg of dry matter (DM) per 100 kg of live weight (LW). Grazing took place at the following phenological stages of ryegrass: Vegetative 1 (June 24-August 6); Vegetative 2 (August 7-September 3); Pre-flowering (September 4-October 4) and Flowering (October 5-21). The criterion to determine the interval between grazing sessions was the accumulated thermal sum (TS) of 250 growing degree-days equivalent to the TS necessary for the emergence of two leaves of ryegrass (CONFORTIN et al., 2010b).

The plot occupation period was 12 days, and one grazing cycle was considered to be the period of plot occupation plus the interval until the start of the next occupation. TS was calculated using the equation: TS = Σ (Dat - 5°C), in which Dat = daily average temperature for the period (°C); and 5°C = value regarded as base temperature for growth of cold-season species.

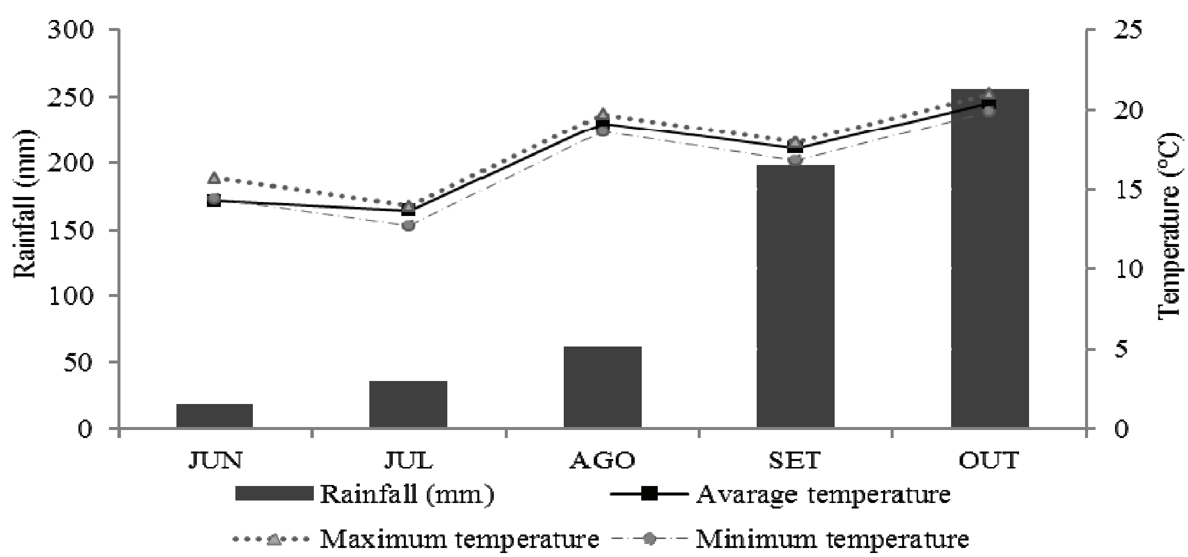

Figure 1. Rainfall (mm), maximum temperature, minimum temperature and average temperature (°C) during the experimental period. Santa Maria, Rio Grande do Sul State, 2012.

The experimental animals were Suffolk female lambs, at eight months old and average weight of 43 ± 4.56 kg. The lambs had free access to water and mineral salt. Three test lambs were used per plot and a variable number of put-and-take animals were used to adjust the intended allowance.

Herbage mass was determined through the direct visual estimate method with double sampling, at the start and end of each grazing period. Samples obtained from the sections provided the levels of dry matter and the share of different botanical and structural components of the pasture, at the start and end of each period. The samples were separated manually into leaf blade, stem and inflorescence of ryegrass and other species. Later, the material was dried in a forced-air oven at 55ºC for 72 hours and weighed. The leaf:stem ratio was determined from the rate of leaves and stems, at the start and end of each grazing period.

The daily herbage accumulation rate was determined from the following formula:

$$DAR = (MF1(i) – MF2(i-1))\ D^{-1}$$

which:

DAR =daily herbage accumulation rate;

MF1 = pre-grazing herbage mass of grazing cycle ‘i’;

MF2 = post-grazing herbage mass of grazing cycle ‘i – 1’,

D = number of days in the interval period between grazing sessions.

The stocking rate was calculated using formula:

$$TL = ((PCt * DP) + (PCr * D))\ DP^{-1}$$

which:

TL = instant stocking rate (kg ha^{-1} of LW);

PCt = live weight of test lambs (kg ha^{-1});

PCr = live weight of put-and-take lambs (kg ha^{-1});

DP = days in the period of pasture occupation;

D = number of days put-and-take lambs remained in the pasture.

Herbage allowance was calculated using formula:

$$OF = ((MF\ n^{-1} + DAR)\ TL^{-1})*100$$

which:

OF = herbage allowance (%);

MF = mean herbage mass (kg ha^{-1} of DM) = [(MF initial + MF final) 2^{-1}];

N = number of days in the grazing period (days);

DAR = daily herbage accumulation rate (kg ha^{-1} day^{-1} of DM);

TL = stocking rate of grazing cycle (kg ha^{-1} of LW). The allowance of green leaf blades was obtained by multiplying the herbage allowance by the mean percentage of leaf blades in MF.

In each plot, 35 tillers were marked with colored rings, distributed in seven locations, and monitored for canopy and pseudostem height. After each grazing cycle, the process of tiller marking was redone to include new individuals, in order to preserve the representativeness of the population. The length of leaf blades (expanded, in expansion and senescent) in these tillers were measured daily during the grazing periods and, using these measurements, it was possible to calculate the mean length of leaf blades, the intensity and defoliation frequency of leaf blades.

Defoliation intensity (%) was measured using the formula: defoliation intensity = ((CLFi-CLFf) $CLFi^{-1}$)*100, in which CLFi and CLFf are, respectively, the initial length and final length of the leaf blade. To estimate defoliation frequency (number of defoliations/blades/day) in marked tillers, senescent or damaged leaf blades were identified with correction fluid the day before the animal entered the pastured. Defoliation frequency was calculated from the formula: defoliation frequency = number of contacts during grazing days / (number of possible contacts * duration of the evaluation). The time interval between two successive defoliations was determined by the formula: time interval = 1 /frequency.

To evaluate the population pattern of tillering, three PVC rings 10 cm in diameter (0.0078 m^2) were used, fixed on the soil in each experimental unit. The demography of tillering was based on identifying and counting remaining live tillers and the emergence of new tillers. The first tiller marking took place at the start of the first grazing cycle, when all tillers of ryegrass within the area demarcated by the rings were marked with plastic wire of the same color, and named as the first generation of tillers (G1). Eighteen days after the first marking, live tillers from the first generation were recounted and new tillers were marked using plastic wire of a different color, and named second generation of tillers (G2) and, thus successively, until the end of the usage period of ryegrass.

The study calculated the rates of tiller emergence (TAP), mortality (TMP) and survival (TSP). The stability index (IESP) of the population of tillers was calculated as per Bahmani et al. (2003), in which: IESP = TSP*(1 + TAP). Site occupation, which measures the relationship between the emergence of leaves and the occupation of leaf shoots to form tillers, was calculated by dividing the tiller emergence rate by the rate of leaf blade emergence. Tiller population density (tillers m^{-2}) was evaluated during the grazing cycles by counting live tillers, at three fixed areas of 0.0625 m^2 per plot, identified with stakes. To determine mass per tiller (g of DM $tiller^{-1}$), samples were cut at three areas similar to those used to evaluate tiller population density. The number of tillers in these samples was quantified; later, those samples were dried and weighed. Dry mass was divided by the number of tillers in the sample.

The experimental design experimental was entirely randomized, with repeated measurements over time, three treatments and two area replications. Analysis of variance was carried out using the 'Mixed' procedure in SAS (2002-2005) version 9.2. A structure selection test was used, following the Bayesian information criterion (BIC), to determine the model that best represented the data. Whenever differences were observed, the averages were compared using the 'lsmeans' procedure. The interaction between treatments and phenological stages was unfolded whenever it was significant at 10% probability, and variable responses were modeled using a polynomial function up to the third order. In the regression analysis, models were chosen based on the significance of the linear and quadratic coefficients, using Student's t-test, at 10% probability. The variables were also subjected to Pearson's linear correlation analysis.

Results and discussion

For the predicted values of 12, 9, and 6 kg of DM 100^{-1} kg of live weight (LW), the observed herbage allowances were 12.6, 8.8 and 6.6 kg of DM 100^{-1} kg of live weight (LW), respectively.

There was no interaction between herbage allowance and phenological stage for the defoliation intensity of leaf blades ($p = 0.3225$). There was a difference between herbage allowances for defoliation intensity ($p = 0.0544$). At herbage allowances of 6 and 9%, as defoliation intensities were similar, averaging 70.8%, which was 22.7% greater than the 54.7% intensity observed at 12% allowance. The defoliation intensity of leaf blades was positively correlated to instant stocking rate ($r = 0.67$; $p = 0.0005$) and negatively correlated to average leaf blade length ($r = -0.77$; $p < 0.0001$) and com a canopy height ($r = -0.84$; $p < 0.0001$).

Defoliation intensity varied according to allowance, whereas Lemaire and Agnusdei (2000) observed constant defoliation intensity of 50-55% for grass species from genus *Lolium* sp. According to those authors, this constant defoliation intensity could be attributed to an adjustment in bite depth to the average leaf blade length. In ryegrass, under different herbage allowances, this adjustment may not have occurred due to the fact that defoliation intensity fit the negative linear regression model because of leaf blade length ($\hat{Y} = 94.8 - 4.20x$; $r^2 = 0.60$; $p < 0.0001$). According to this regression, a one-cm increase in leaf blade length caused a 4.20% reduction in defoliation intensity. Thus, the lower defoliation intensity at 12% allowance is accompanied by greater leaf blade length and taller canopy height (Table 1).

Defoliation intensity was greater at the pre-flowering stage (72.6%), when compared to the Vegetative 1 and Flowering stages (60.5%) and did not differ from Vegetative stage 2 (68.8%). Vegetative stage 1 had greater leaf blade length, higher final leaf:stem ratio and tiller population density, and lower stocking rate (Table 1), which provided animals greater opportunity to select leaf blades, resulting in low defoliation intensity. At Vegetative stage 2, structural changes in the canopy – such as lower height, tiller population density and final leaf:stem ratio (Table 1) – caused lambs to develop grazing strategies that did not differentiate this intensity from those observed at the other stages. According to Jochins et al. (2010), whenever the entire diet of animals consists of pasture, alterations in herbage availability result in changes to certain components of intake behavior, such as bite rate and grazing time. As such, these changes can affect defoliation intensity.

Table 1. Canopy height, instant stocking rate, leaf blade length, final leaf:stem ratio and tiller population density of Italian ryegrass managed under different herbage allowances and phenological stages.

	Canopy height[1]	Instant stocking rate[2]	Length leaf blades[3]	Final leaf: stem ratio	TPP[4]
		Herbage allowances[5]			
6%	7.4b	3425a	5.6b	1.3	4926
9%	9.6ab	2266b	6.5b	1.2	4735
12%	13.2a	1841c	8.6a	1.5	5029
CV(%)	8.15	3.46	12.57	17.3	20.9
		Phenological stages			
Vegetative 1	11.2a	1572c	9.0a	3.0a	5955a
Vegetative 2	8.3b	2457b	7.1b	1.6b	5336b
Pre-flowering	9.1b	3125a	5.8c	0.47c	4589c
Flowering	11.7a	2889a	5.7c	0.30c	3705d
CV(%)	10.2	3.99	17.0	15.1	20.5
		Significance of (p) effect			
Herbage Allow.	0.0949	0.0021	0.0617	0.7490	0.6971
Phenol. Stages	0.0390	< 0.0001	< 0.0001	< 0.0001	0.0004
Interaction	0.5115	0.1609	0.2186	0.5853	0.2713

Values followed by letters, in the columns, differ from one another by the *lsmeans* test at 10% significance; [1]canopy height (cm); [2]instant stocking rate (kg of LW ha^{-1}); [3]leaf blade length (cm); [4]tiller population density (tillers m^{-2}); [5]Herbage allowances: 6, 9, or 12 kg DM 100^{-1} kg LW.

At the Pre-flowering stage, defoliation intensity may likely be explained by the value of instant stocking rate, which was higher (Table 1). According to Lemaire and Chapman (1996), under an intermittent grazing method, defoliation intensity depends directly on the grazing period and stocking density. Therefore, higher instant stocking rates can cause greater defoliation intensities. In *Lolium perenne*, managed with stocking rates of 29, 77 and 91 sheep/hectare, Hodgson (1990) observed defoliation intensities ranging between 13% at the lowest stocking rate and 67% at the highest stocking rate.

Defoliation intensity was lower in the Flowering stage, even though the instant stocking rate, final leaf:stem ratio and leaf blade length were similar to those observed during the stage Pre-flowering stage (Table 1). During this stage, due to greater sward height and lower tiller density (Table 1), lambs may not have able to adjust intake behavior to maintain their intake of leaf blade, given the larger share of stems in the diet.

There was no interaction between herbage allowance and phenological stage for the interval between defoliations ($p = 0.7258$). A difference was found between herbage allowances for the interval between defoliations ($p = 0.0076$), with values of 1.19, 1.50, and 2.01 days until returning to the same tiller at allowances of 6, 9 and 12%, respectively, and these differed from one another.

The intervals observed between defoliations indicate that lambs grazed 84.0, 66.7 and 49.7% of the total area daily, when subjected to allowances of 6, 9 and 12%, respectively. By dividing the area grazed daily by the average number of lambs during pasture usage, it is calculated that each lamb grazed daily over 116.2, 143.7, and 165.8 m^2 at the allowances of 6, 9 and 12%, respectively. The interval between defoliations was negatively correlated to instant stocking rate ($r = -0.79$; $p = 0.0001$).

Thus, the highest instant stocking rates, at 6% allowance, resulted in greater used of the total grazed area and, consequently, in a smaller each for each lamb to graze.

The interval between defoliations was greater (1.76 days) in Vegetative stage 1; intervals were similar for the Pre-flowering and Flowering stages, and lower than the other stages (1.44 days). The interval between defoliations during Vegetative 2 was similar to the other stages (1.62 days). The interval between defoliations was positively correlated to final leaf:stem ratio ($r = 0.51$; $p = 0.0188$; Table 1). The transition from Vegetative stage 1 and 2 to the Pre-flowering and Flowering stages de caused structural changes in the canopy, such as lower final leaf:stem ratio, leaf blade length and tiller population density (Table 1). These changes, in addition to hindering the defoliation process, may have resulted in lower diet quality. As a result, in order to meet their nutritional needs during the Pre-flowering and Flowering stages, lambs altered their defoliation strategies by return to the same leaves over a shorter interval of days.

There was no interaction between herbage allowance and phenological stage, nor was there any difference between evaluated herbage allowances ($p > 0.1$) for rates of tiller emergence, survival and mortality, or for the stability index the tiller population.

The tiller emergence rate did not differ between phenological stages ($p = 0.3928$; Figure 2), averaging 89.3%. This may indicate an adaptation of ryegrass to its use under different herbage allowances.

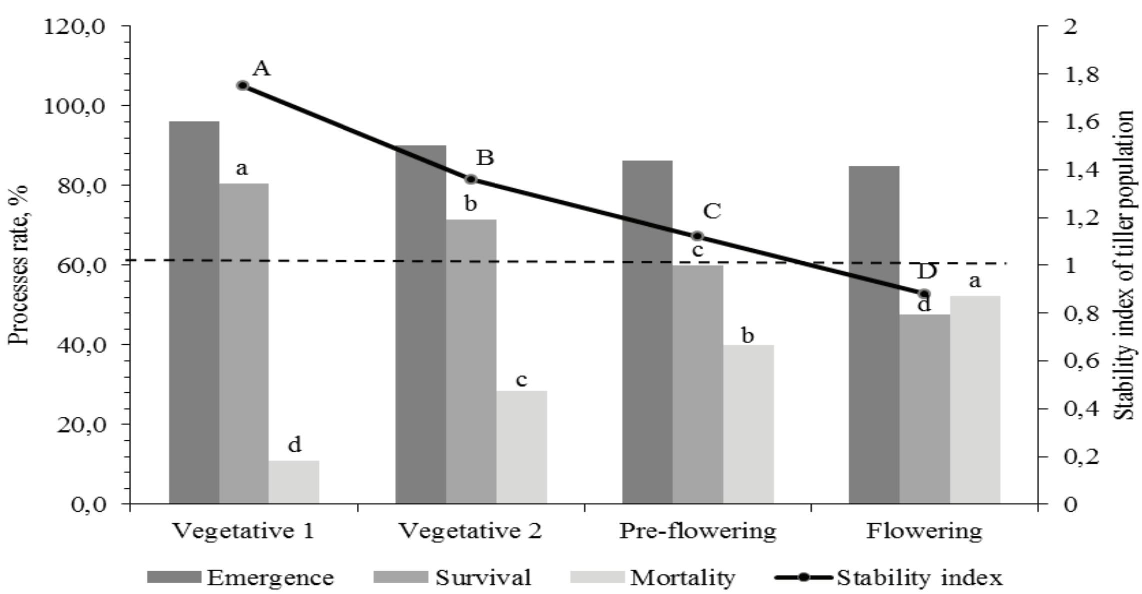

Figure 2. Rates of tiller emergence, survival and mortality (%) and stability index of the tiller population, in ryegrass, as a function of phenological stages. Different letters indicate that averages differ from one another ($p < 0.1$).

According to Matthew et al. (2000), the tiller emergence rate can be regarded as an adaptive strategy by plants to grazing, in order to restore leaf area. As such, the tiller emergence rate and its lifespan are important characteristics for the persistence of plants in the community (ELYAS et al., 2006).

Tiller mortality rate was different in all phenological stages ($p = 0.0001$; Figure 2), increasing between Vegetative stage 1 to the Flowering stage, with values ranging from 10.9 to 52.2%, respectively. The tiller survival rate differed between phenological stages, with inverse behavior as tiller mortality rate ($p = 0.0001$; Figure 2), ranging from 89.1 to 47.8%, respectively. Tiller mortality rate was negatively correlated to final leaf:stem ratio ($r = - 0.79$; $p < 0.0001$). The reduction in the leaf:stem ratio stem (Table 1) as phenological stages advanced may have caused a decrease in the supply of photoassimilates, which in turn may have contributed to higher tiller mortality. According to Lemaire and Chapman (1996), the main cause of tiller mortality is the removal of the apical shoot by animal grazing, which occurs especially during the reproductive period when apical shoots are situated higher up the plant from stem growth towards the defoliation horizon, representing another reason for greater tiller mortality in this occasion.

The stability index of the tiller population (IESP) differed between grazing cycles (Figure 2) and fit the linear regression model $\hat{Y} = 2.57 - 0.0009\,x$ ($r^2 = 0.78$; $p = 0.0001$) as a function of the thermal sum accumulated during pasture use. Thus, each one-degree increase in the accumulated thermal sum caused a reduction of 0.0009 points in the stability index of the tiller population. According to Caminha et al. (2010), the IESP allows an integrated analysis of changes in the population, as it contemplates emergence and tiller survival rates jointly, not individually, which favors the visualization of the effect of environmental and management factors on the pasture, allowing for better understanding and management.

Despite the decrease in the stability of the tiller population, caused especially by higher tiller mortality, this variable was lower than 1 (Table 1) only during the Flowering stage. Barth Neto et al. (2013) also observed stability rates below 1, at the end of the usage period of ryegrass, in areas under intermittent grazing with prior soybean crop. According to Bahmani et al. (2003), the response obtained during this stage means that ryegrass showed that the tiller emergence rate was lower than tiller survival rates over the same period of time.

There was no interaction between herbage allowance and grazing cycle for tiller population density. Herbage allowances did not influence tiller population density, averaging 4896 tillers m^{-2}. Confortin et al. (2013) did not observe any variation in ryegrass tiller density, either, when evaluating different herbage masses, finding an average of 2919.2 tillers m^{-2}. For his part, Cauduro et al. (2006) observed that low grazing intensity (5 times the potential intake of lambs) contributed to the fact that tiller population density was 9.21% lower than the density of 3235.6 tillers m^{-2} observed at moderate intensity (2.5 times the potential intake of lambs). The higher intensity of leaf blade removal, at the 6% and 9% allowances, may not have caused a sufficient opening in the herbage canopy to increase light incidence over basal shoots, and consequently there was no increase in new tiller production.

Tiller population density differed between the different grazing cycles (Table 1) and fit the linear regression model ($\hat{Y} = 8215.76 - 2.24x$; $r^2 = 0.66$; $p < 0.0001$) as a function of the thermal sum accumulated during pasture use, with a reduction of 2.24 tillers m^{-2} for every degree accumulated during the grazing cycles. According to Lemaire and Chapman (1996), a tiller density on a given pasture is due to the balance between mortality rates and the emergence of tillers. The negative correlation ($r = - 0.82$; $p < 0.0001$) between population density and tiller mortality rate indicates that the reduction in tiller density as the grazing cycle advanced may have occurred due to tiller mortality, given that there was no variation in the tiller emergence rate (Figure 2). Nor were environmental variables such as light availability, water, temperature and nutrients limiting.

There was an interaction between herbage allowance and phenological stage for mass per tiller ($p = 0.0074$). In Vegetative stage 1, mass per tiller was similar between all evaluated allowances, averaging 0.032 grams. In the Vegetative 2, Pre-flowering and Flowering stages, mass per tiller was similar at 6 and 9% allowances, with values of 0.041, 0.039, and 0.038 grams, respectively; this mass was lower than those observed in 12% allowance herbage. At 12% allowance, mass per tiller was 0.07, 0.069, and 0.075 grams for the Vegetative 2, Pre-flowering and Flowering stages.

The results obtained for mass per tiller, at the Vegetative 2, Pre-flowering and Flowering stages, at 6 and 9% allowances are in agreement with statements by Sbrissia and Da Silva (2008) that frequent and severe defoliation leads to lower individual tiller weight.

There was an interaction between herbage allowance and vegetative stage for site occupation ($p = 0.0985$). At the Vegetative 1 and 2 and Flowering phenological stages, site occupation was 62.6, 17.3, and 19.3%, respectively. During the Pre-flowering stage, site occupation differed between the different evaluated allowances. Site occupation was greater at 12% allowance, intermediate at 9% allowance 9% and lower at 6% allowance, with values of 34.2, 25.5, and 20.6%, respectively.

Site occupation was positively correlated to the number of live leaves ($r = 0.47$; $p = 0.0194$). There was an interaction between herbage allowance and vegetative stage ($p = 0.0016$) for the number of live leaves, with no difference between Vegetative 1 and 2 and Flowering phenological stages. The number of live leaves was greater, intermediate and lower, respectively, at the Pre-flowering stage, at 12, 9, and 6% herbage allowances, with values of 4.0, 3.4, and 2.9, respectively. The link between site occupation and number of live leaves is essential to understanding the tillering process, as each leaf blade has an axillary shoot that can originate a new tiller and site occupation measured the rate of leaf shoots that can evolve into tillers. Therefore, site occupation is essential to produce pasture, and is associated with the opportunity by animals to consume more or less herbage, of greater or lower quality, therefore ultimately relating it to animal performance. Thus, changes for site occupation at the different allowances occurred only when structural changes began in the pasture towards the reproductive phase, which indicates that the management implemented did not influence the site occupation of ryegrass at the other stages.

Conclusion

Structural changes in the ryegrass pasture caused by management with low herbage allowances and/or by successive phenological stages altered the defoliation strategies of lambs in that they graze intensely and frequently. Ryegrass pastures managed under different herbage allowances did not show variations in the rates of emergence, mortality and tiller survival, but these variables are affected by the different phenological stages. At the start of the reproductive phase of ryegrass, high herbage allowances lead to greater site occupation.

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