

Morphogenic, structural characteristics and population stability index of ryegrass tillers submitted to stocking methods

[Características morfológicas, estruturais e índice de estabilidade populacional de perfilhos de azevém submetidos a métodos de lotação]

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ABSTRACT

This study aimed to evaluate the morphogenic and structural characteristics and the population stability index of ryegrass (*Lolium multiflorum* Lam.) tillers when the pasture was submitted to two stocking methods during grazing cycles. The experimental design was a complete randomized block, with repeated measurements over time (n=6 cycles), two treatments (stocking methods) and three area replicates. In the continuous stocking method, the highest population density of tillers was observed. The highest tiller weight occurred in the rotational stocking method. The morphogenic variables and the other structural variables were not altered by the stocking methods and showed differences during the grazing cycles. The tiller population stability index was similar in the two pasture management strategies, and both can be used for ryegrass management considering this parameter.

Keywords: tiller of stability, *Lolium multiflorum* Lam., continuous stocking, rotational stocking

RESUMO

Este estudo teve como objetivo avaliar as características morfológicas, estruturais e o índice de estabilidade populacional de perfilhos do azevém (*Lolium multiflorum* Lam.) quando a pastagem foi submetida a dois métodos de lotação, durante ciclos de pastejo. O delineamento experimental foi em blocos completos casualizados, com medidas repetidas ao longo do tempo (n = 6 ciclos), dois tratamentos (métodos de lotação) e três repetições de área. No método de lotação contínua, foi observada a maior densidade populacional de perfilhos. O maior peso de perfilhos ocorreu no método de lotação rotativa. As variáveis morfológicas e as demais variáveis estruturais não foram alteradas pelos métodos de lotação e apresentaram diferenças durante os ciclos de pastejo. O índice de estabilidade populacional de perfilhos foi semelhante nas duas estratégias de manejo do pasto e ambas podem ser utilizadas para o manejo do azevém considerando-se esse parâmetro.

Palavras-chave: estabilidade de perfilhos, *Lolium multiflorum* Lam., lotação contínua, lotação rotativa

INTRODUCTION

The livestock activity in Brazil is based on pasture utilization as the main food resource and, in the Southern states, the most utilized species is Italian ryegrass (*Lolium multiflorum* Lam.) due to its productive potential and good adaptation to environmental conditions in that region. The adoption of pasture management strategies aimed at maximizing both plant and animal production

are extremely important. The adoption of different ways to conduct stocking, continuously or rotationally, are among these strategies.

In continuous stocking, animals have unlimited and uninterrupted access to the entire area to be grazed during the entire grazing season and in rotational stocking, alternation between defoliation and rest periods occurs. Due to the intercalation of rest and grazing periods, in rotational stocking, the regrowth process occurs

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Submitted: May 22, 2022. Accepted: August 18, 2022.

in isolation from the grazing process. In this context, it is recommended that defoliation management be carried out using degree-days as it allows a direct association with the morphogenic characteristics of forages plants (Ongaratto *et al.*, 2020). On the other hand, continuous stocking is characterized by milder changes in the sward condition over the period.

Both stockings affect the adaptive responses of forage plants differently. The persistence strategies of the plants under different defoliation methods can be accessed by studying individual tillers in the plant community. This information allows the identification of management tools that result in canopies with a favorable structure for plants and animals (Carvalho *et al.*, 2001).

In this context, the study of the morphogenic variables associated with the tiller stability index becomes important in the comparison of stocking methods. In winter grasses, published data evaluating stocking methods are limited to information on morphogenic characteristics and other pasture variables (Cauduro *et al.*, 2006, 2007; Barth Neto *et al.*, 2013). Considering tiller population stability, research has been carried out with a focus on different grazing practices (Graminho *et al.*, 2014; Stivanin *et al.*, 2014; Duchini *et al.*, 2017).

However, it is necessary to jointly evaluate pasture growth through its morphogenic characteristics when associated with plant population stability in different grazing methods. This favors the estimate of the effect of environment and management factors on the pasture, allowing a better understanding and manipulation of the processes involved (Bahmani *et al.*, 2003; Caminha *et al.*, 2010; Sbrissia *et al.*, 2010). The objective of this study was to evaluate the morphogenic and structural characteristics and the stability index of the tiller population of ryegrass (*Lolium multiflorum* Lam.) when the pasture was submitted to two stocking methods.

MATERIALS AND METHODS

The experiment was approved by the Ethics Committee for Animal Experimentation of Universidade Federal de Santa Maria (UFSM), Protocol 9708210518. The experiment was performed from July to November 2019 at the Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil. Climate, according to Köppen's classification, is humid subtropical. The meteorological data for the months that comprised the experimental period were obtained from the UFSM Meteorological Station (Fig. 1).

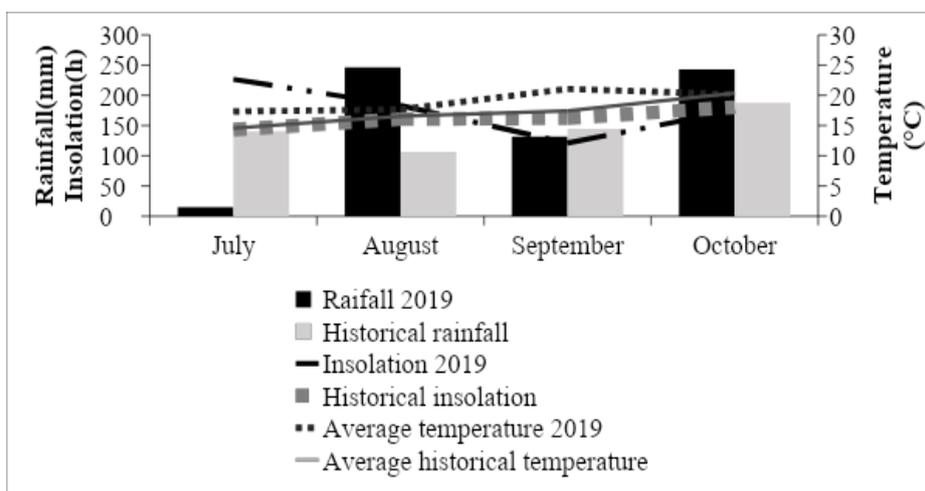


Figure 1. Average monthly rainfall (mm), insolation (h) and temperature (°C) from July to October 2019 and normal historical data. Santa Maria/RS.

The experimental site had an area of 4.8 ha, divided into six paddocks and of those six, three were subdivided into five plots of 0.16 ha. The ryegrass pasture (*Lolium multiflorum* Lam.) was

established in May 2019. The soil is Paleudalf and, in the experimental area, had the following average ratios: pH-H₂O = 5.2; % clay = 26; P = 8.1mg L⁻¹; K = 85.6mg L⁻¹; % OM = 2.5m V⁻¹;

$Al^3 = 0.7 \text{ cmolc L}^{-1}$; $Ca^{+2} = 3.6 \text{ cmolc L}^{-1}$; $Mg^{+2} = 1.6 \text{ cmolc L}^{-1}$; CTC pH7 = 11.4. The fertilizer consisted of 200 kg ha^{-1} of the formula 05-20-20 (N-P-K) and 115 kg ha^{-1} of N in urea form split into three applications.

We evaluated two grazing management strategies: continuous stocking - keeping forage mass between $1.200\text{-}1.600 \text{ kg DM ha}^{-1}$ and rotational stocking - with post-grazing target sward height of $10 \pm 1 \text{ cm}$. Criterion utilized to determine the rest period for rotational stocking was the thermal sum (TS) of $187.5 \text{ degree-days (DD)}$, equivalent to 1.5 Italian ryegrass phyllocron value (Confortin *et al.*, 2010). Thermal sum (TS) was calculated by the equation: $TS = \sum(Tmd) - 5$, where: Tmd is the daily average temperature of the stocking cycle; the value of 5 grade is the minimum temperature required for growth of cool season forage species.

Experimental animals were Angus heifers with initial age and body weight (BW) of eight months and $157 \pm 3.94 \text{ kg}$, respectively. Three test animals (permanent animals during all stocking period) were used per experimental unit. For the maintenance of post-grazing canopy height in rotational stocking and forage mass in continuous stocking, a variable number of put-and-take animals were used. The pasture utilization comprised six stocking cycles (1-Jul./07 to Aug./13; 2-Aug./14 to Sep./02; 3-Sep./03 to Sep./17; 4-Sep./18 to Oct./02; 5-Oct./03 to Oct./17; 6-Oct./18 to Nov./01). Morphogenic measurements were performed in cycles 1 to 4 and stability index of the tiller population from 1 to 6. The duration of stocking cycles, in days, was similar for both stocking methods.

For every stocking cycle 20 measurements of canopy height per experimental unit (pre- and post-grazing in rotational stocking) were taken. In continuous stocking, forage mass (FM, kg DM ha^{-1}) was determined by visual estimation technique with double sampling. For rotational stocking, FM was determined by two herbage mass samples taken at ground level every pre- and post-grazing using frames of 0.25 m^2 . These places were representative of the paddock average canopy height. The forage from cuttings were dried at $55 \text{ }^\circ\text{C}$ for 72 h. The forage allowance (FA; kg DM kg BW^{-1}) was calculated

in each stocking cycle according to the methodology of Sollenberger *et al.* (2005). The stocking rate (kg BW ha^{-1}), was calculated by adding the average weight of the test animals to the average weight of each put and take animal, multiplied by the number of days that it remained on the paddock divided by the number of days of the stocking cycle. The instantaneous stocking rate, in the rotational stocking, was calculated by the quotient between the heifer's live weight and the area of the plot occupied by them.

Morphogenetic and structural characteristics were evaluated in 18 tillers per experimental unit, by of marked tillers. Every beginning of a new stocking cycle, a new group of tillers was selected for evaluation. In continuous stocking and in the rest periods in rotational stocking measurements were made twice weekly. In rotational stocking the measurements were made daily when the plot was being grazed. In these occasions, the length of fully expanded, expanding, and senescent leaf blades, canopy height, and height of pseudostem were measured in cm. From these measurements, the following variables were calculated: leaf appearance rate (degree-days), leaf expansion rate ($\text{cm degree-days tiller}^{-1}$), leaf senescence rate ($\text{cm degree-days tiller}^{-1}$), leaf elongation duration (degree-days), leaf lifespan (degree-days), phyllochron (inverse of the rate of appearance of the leaves (degree-days) and number of live leaves (Lemaire and Chapman, 1996).

The tiller population density and tiller population stability index (IS) variables were evaluated by monitoring tiller generations. The first generation of tillers was tagged with plastic wires of same color, in two fixed frames (0.0078 m^2) in each experimental unit, at the starting of first stocking cycle. In this occasion, the number of tillers of this generation was similar ($P > 0.10$) in all paddocks. Each new grazing cycle the living tillers tagged in the previous generation were counted again and untagged tillers (new tillers) were tagged with plastic wires of a different color. The dynamics of tillering was carried out from the identification and counting of the remaining living tillers and the appearance of new tillers. With the sum of the number of tillers belonging to each generation, it was possible to calculate the density of tillers in each generation (tillers m^2). We calculated rates of tiller appearance (RTA), mortality (RTM) and survival

(RTS; tiller tiller⁻¹ m⁻²) and population density of tillers (PDT; tillers m⁻²). The population stability index (SI) of tillers was calculated according Bahmani *et al.* (2003), in which: SI = RTS*(1+RTA). To determine weight per tiller (g DM tiller⁻¹), cuts were made in two areas (0.0625 m²). The number of tillers in these areas was quantified and, subsequently, these tillers were dried in a circulating air oven at 55°C for 72 h and after weighed. The dry mass value was divided by the number of tillers in the sample.

A randomized complete block following a repeated measures arrangement (n= 4 stocking cycles for morphogenetic variables or n= 6 for tillering variables) was used, with two stocking management strategies (continuous and rotational) and three area replications (paddocks). To compare the stocking systems, the variables with normal distribution were evaluated considering the fixed effects of stocking systems, stocking periods and their interactions and the random effects of blocks, residuals and paddocks nested in stocking systems using the Mixed. We performed a structure selection test, following the Bayesian Information Criterion (BIC) to determine the model that best fit the data. When not fitted to regression models, the mean values were

compared using the ‘lsmeans’ procedure. The interaction between stocking systems and stocking cycles was broken down, when significant at 5% probability. The variables were also analyzed using Pearson correlation analyses.

RESULTS

Meteorological data of the experimental period showed that the average values of temperature, rainfall and insolation were 11.4%, 15.6% and 10.9% higher in relation to the historical averages (Fig. 1).

There was no interaction (P>0.05) between stocking methods × stocking cycles for forage mass, stocking rate and forage allowance variables. The forage mass was similar in paddocks used to evaluate stocking methods (Table 1). The forage mass differed between stocking cycles (P<0.10) being higher in the sixth (1654.6±117.0kg DM ha⁻¹), intermediate in second and fifth cycles (1512.2±117.0 kg DM ha⁻¹) and lower for the other cycles (1414.0±117.0kg DM ha⁻¹). The post-grazing canopy height mean value in the rotational stocking was 11.5±0.7cm. The average canopy height in continuous stocking was 14.9±2.0cm.

Table 1. Morphogenetic and structural characteristics, phyllocron of Italian ryegrass was submitted to two stocking method

Variables	Stocking		SEM	P-value
	Continuous	Rotational		
Forage mass, kg DM/ha	1529	1445	103.7	0.1371
Stocking rate, kg/ha BW	1062	1259	112.6	0.0607
Leaf appearance, cm/degree-day	0.008	0.007	0.0003	0.2448
Phyllocron, degree-day	143.8	148.2	5.33	0.2254
Leaf expansion, cm/degree-day	0.054	0.051	0.007	0.7052
Leaf lifespan, degree-day	505.8	505.7	19.72	0.9309
Number of live leaves	3.69	3.58	0.10	0.3228
Leaf senescence, cm/degree-day	0.038	0.032	0.003	0.2632

SEM= Standard error of mean.

The grazing heifers, in both stockings, were submitted to the same forage allowance (1.34±0.15kg DM kg BW⁻¹; P = 0.4680). The stocking rate was 18.5% higher in rotational compared to the continuous stocking (Table 1) and did not differ between stocking cycles (P>0.10; 1188.4±149.9kg ha⁻¹ of BW). The instantaneous stocking, in rotational stocking, presented an average value of 7819.5±1323.8kg ha⁻¹ of BW.

There was no interaction (P>0.05) between stocking method × stocking cycles for the variables leaf appearance rate, phyllochron, leaf lifespan, leaf senescence rate, leaf expansion rate and number of live leaves. These variables did not differ between stocking methods (Table 1). There was a difference between stocking cycles (P<0.10) for leaf appearance rate, phyllochron, leaf senescence rate, leaf expansion rate and number of live leaves. The leaf appearance rate

was higher and similar in grazing cycles 1 and 2 (0.008 ± 0.003 leaf degree-days⁻¹), and lower in 3 and 4, similar to each other (0.007 ± 0.003 leaf degree-days⁻¹). The phyllochron was smaller in grazing cycles 1 and 2 and similar to each other (137.6 ± 5.8 degrees-day) and greater (154.55 ± 5.86 degrees-day) in 3 and 4 that did not differ between themselves.

The leaf senescence rate was higher and similar in grazing cycles 1 and 2 (0.043 ± 0.005 cm degree-days⁻¹), lower and similar to each other in cycles 3 and 4 (0.025 ± 0.005 cm degree-days⁻¹). The leaf expansion rate was higher and similar in grazing cycles 1 and 2 (0.069 ± 0.007 cm degree-days⁻¹), lower in cycles 3 and 4 (0.033 ± 0.007 cm degree-days⁻¹). There was no difference between generations of tillers for leaf lifespan (504.8 ± 19.7 degree-days; $P > 0.10$). The number of live leaves was adjusted to the decreasing linear regression model in relation to the thermal sum ($\hat{Y} = 4.3725 - 0.0016x$; $r^2 = 0.57$; $P = < 0.001$; $CV = 8.20$).

There was interaction ($P < 0.05$) between stocking methods \times stocking cycles for pseudo-stem height. In the stocking cycle 4, there was a difference between the stocking methods for pseudo-stem height, being greater in continuous stocking method (6.2 ± 0.32 cm; $P = 0.0221$) in relation to rotational (5.2 ± 0.32 cm). In the other

stocking cycles the height of the pseudo-stem was similar ($P > 0.05$) for both methods, being 4.1 ± 0.32 cm in cycle 1, 4.34 ± 0.32 cm in cycle 2 and 4.8 ± 0.32 cm in cycle 3.

There was no interaction ($P > 0.05$) between stocking methods \times stocking cycles for tiller population density and tiller weight. There was a difference between stocking methods ($P = 0.0238$), with tiller population density being 13 % higher in the continuous (2176.1 ± 74.8 tillers m²) than in the rotational stocking (1919.0 ± 74.8 tillers m²). Tiller weight was 14% higher in the rotational stocking ($P < 0.10$; 0.033 ± 0.002 g) than in the continuous stocking (0.029 ± 0.002 g).

There was no interaction ($P > 0.05$) between stocking methods \times stocking cycles for the tiller population stability index. There was a difference between tiller generations ($P < 0.10$) for the tiller population stability index. The stability index of tillers (Figure 2) was higher and equal ($P > 0.05$) in G2 and G3 generations (1.66 ± 0.1), the G5 generation (1.13 ± 0.1) did not differ from the generation G4 (1.28 ± 0.1) and G1 and G6 generations were smaller and similar to each other (0.96 ± 0.1).

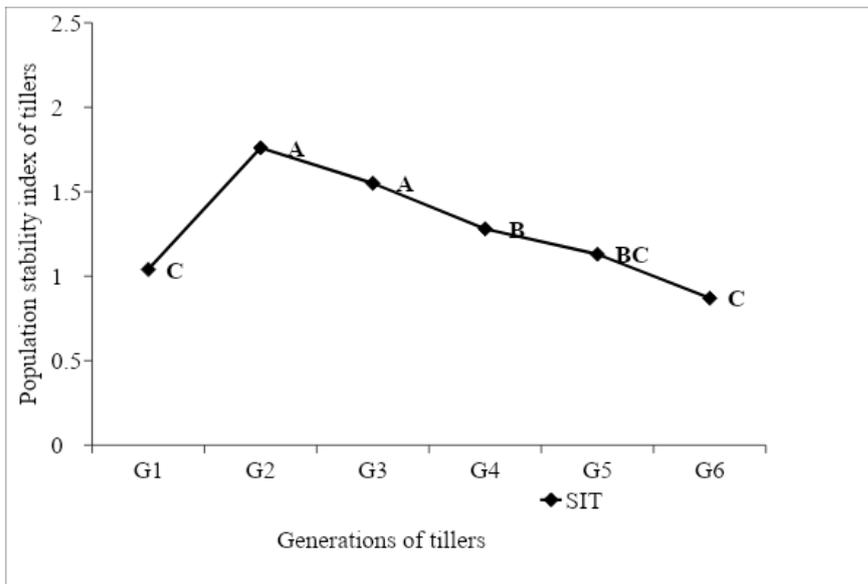


Figure 2. Stability index of tiller population (uppercase letters in bold), in Italian ryegrass, as a function of generations of tillers: G1 – 07/26/2019; G2 - 08/17/2019; G3 - 9/07/2019; G4 - 09/20/2019; G5 - 10/04/2019; G6 - 10/18/2019. Different letters indicate that the means differ from each other by the ls means procedure ($P < 0.10$).

DISCUSSION

The stocking practices alter the pasture structure and the leaf area index, which, in turn, alters the generation and expansion of new tissues (Duchini *et al.*, 2017). However, it is important to show that these alterations affect, firstly, the structural characteristics, highlighting the changes that involve the tiller population (Lemaire and Chapman, 1996). According to Cauduro *et al.* (2006), stocking practices influence morphogenic characteristics (continuous stocking results in higher rates of leaf appearance and elongation and leaf lifespan in the first stocking cycle) and structural characteristics, with the highest tiller population density observed in continuous stocking. The information should be viewed with caution, as the authors describe that the beginning of the experimental protocol occurred when the ryegrass pasture was at an advanced phenological stage.

The similarity observed for the morphogenic traits, leaf appearance rate, phyllochron, leaf expansion, lifetime of ryegrass leaves in different stocking practices can be explained because these variables are genetically determined, although they may be influenced by biotic and abiotic (Lemaire *et al.*, 2009). The highest rate of leaf appearance occurred during the vegetative stage, which is characterized as favorable to plant development (Lemaire and Chapman, 1996). Leaf appearance rate was negatively correlated with pseudostem height ($p = 0.002$; $r = -0.69$). The increase in pseudostem height is associated with the greater length that the leaf blade needs to travel before being emitted in the canopy (Duru and Ducrocq, 2000).

The phyllochron was lower in the initial stages of ryegrass development (cycles 1 and 2) and according to Lemaire *et al.* (2008), this condition is associated with the greater efficiency with which the grass intercepts and converts light energy in leaf tissue. The increase in phyllochron with the approach of the reproductive stage was significant and expected, since at this stage the plant allocates most of the nutrients to the formation of the reproductive structure, considerably reducing the production of new leaves (Cauduro *et al.*, 2006; Duchini *et al.*, 2017).

The similarity of leaf expansion occurred, possibly because forage mass and phyllochron value were also similar for both stockings (Table 1). The leaf expansion seems to be the morphogenic variable that, in isolation, most directly correlates with the forage dry mass (Confortin *et al.*, 2010). The lowest rate of leaf expansion from the third grazing cycle is related to the phenological stage of ryegrass at this time, allocating photo assimilates primarily to reproductive structures and reducing expansion rate (Duru and Ducrocq, 2000).

The lifespan of leaves was similar between stocking practices and stocking cycles. The variable is dependent on the number of live leaves and the phyllochron, which also remained similar during the experimental protocol. It confirms the similarities in maximum forage production potential for the tested stocking practices. The lives lifespan was on average 505.5 degree-days, which was lower than that obtained when ryegrass was managed under rotating stocking (557.3 degree-days) (Confortin *et al.*, 2010). If all tiller leaves had been removed, the potential ceiling of annual ryegrass growth (lifespan of leaves) would occur 40 days after the start of the stocking cycle.

Even though the number of live leaves is a stable genotypic characteristic for a given grass species (Martuscello *et al.*, 2015), with an increase of one degree in the thermal sum accumulated during pasture use, there was a reduction of 0.0016 in the number of live leaves. The reduction in the number of live leaves as the stocking cycle progresses is probably due to the translocation of nutrients to form the reproductive structure at the expense of foliar production (Cauduro *et al.*, 2006). The number of live ryegrass leaves agrees with that described in the literature, between three and four live leaves per tiller (Confortin *et al.*, 2010).

Leaf senescence is a natural process that characterizes the last stage of leaf development, which begins after its complete expansion. The senescence of a leaf in a tiller starts shortly after reaching the balance between the rate of appearance and senescence to keep the number of live leaves per tiller constant (Confortin *et al.*, 2010). The highest value for the leaf senescence rate was concentrated during the initial stage of ryegrass development (cycles 1 and 2) and may

be associated with a higher rate of leaf appearance.

The higher pseudostem height in continuous stocking (cycle 4) is probably a result of increased competition for light between tillers. In this condition, the plant prioritizes the allocation of carbon for the extension of the internodes, to position the young leaves in the upper stratum of the forage canopy (Lemaire, 2001). Stem elongation occurs during the flowering season, and in the present study this change in pasture structure seemed not be a barrier to defoliation, as mentioned by Roman *et al.* (2007) in ryegrass.

For the tiller population to be considered stable, there must be a balance between death and tiller emergence (Lemaire and Chapman, 1996; Sbrissia *et al.*, 2010). This condition can be monitored from the tiller population density (Matthew and Sackville-Hamilton, 2011). In continuous and rotational stocking, the highest (2176.1 tillers m⁻²) and the lowest tiller population density (1919.0 tillers m⁻²) were obtained, respectively. The results were lower than those obtained by Cauduro *et al.* (2006) in continuous stocking (3684.80 tillers m⁻²) and rotating stocking (2666.18 tillers m⁻²). The lower tiller population density in the rotating stocking was expected, due to the presence of a rest period, which determines a free growth of the plants in the absence of grazing. In this way, the plant allocates the reserves and production of photoassimilates to form leaves and reproductive structures of the main stem, reducing tiller production (Cauduro *et al.*, 2007).

The inverse relationship between tiller population density and tiller weight was observed (Calsina *et al.*, 2012). The highest tiller population density observed in continuous stocking is associated with lighter tillers (0.029 g), the opposite being evidenced for rotating stocking (0.033g). It is evident that the variable tiller population density allowed ryegrass greater flexibility and adaptation to the stocking practices employed. Changes at the tillering pattern allow the plant to adapt more quickly to environmental and management conditions when compared to changes in morphogenic and other structural characteristics (Lemaire and Chapman, 1996; Duchini *et al.*, 2017).

On the other hand, the tiller population can be analyzed in more depth, considering information related to tiller generations (Matthew and Sackville-Hamilton, 2011), as well as the tiller stability index. Thus, a low tiller population alone should not be considered an indicator of loss of productive potential and reduced plant persistence, since the pasture can be stable even with low tiller population (Sbrissia *et al.*, 2010).

From the monitoring of the stability index of the tiller population, it is possible to infer about the stability of plants in the pasture. In the sward the lower population density of tillers for rotating stocking is according with the literature consulted (Lemaire and Chapman, 1996; Caminha *et al.*, 2010). It is important to emphasize, however that defoliation management based on rest periods per degree-days may have contributed favorably to the maintenance of the tiller population stability index, since the adopted practice allows a direct association with the tiller's morphogenic (Ongaratto *et al.*, 2020).

The evaluation of the pasture stability index allows the joint assessment of tiller appearance and survival rates, that is, the replacement capacity of dead tillers (Bahmani *et al.*, 2003; Duchini *et al.*, 2017). According to Bahmani *et al.* (2003), stability values below 1.0 indicate unstable pastures. The tiller population stability index was less than 1.0 in the G1 and G6 generations. In the G1 generation, the lowest value for the tiller population stability index may be related to the initial stage of ryegrass development. In the G6 generation, the lowest value may be associated with the end of the ryegrass cycle, which, being an annual forage species, was in the flowering stage (Sbrissia *et al.*, 2010). The results show that the highest rates of tiller stability occurred in generations 2 and 3, and in G2 the value in was numerically higher than in G3. This information it is related to the higher rate of leaf appearance during cycle 2, as each emerged leaf has the potential to originate new tillers (Duchini *et al.*, 2017, 2018). The results suggest that regardless of the management practice adopted, in most of the evaluated period, it was possible to observe the renewal capacity of ryegrass tillers (higher rate of tiller emergence in relation to tiller death), which is confirmed by the index of stability.

CONCLUSION

In the continuous stocking method, the highest population density of tillers was observed. The highest tiller weight occurred in the rotational stocking method. The morphogenic variables and the other structural variables were not altered by the stocking methods and showed differences during the grazing cycles. The tiller population stability index was similar in the two pasture stocking strategies, and both can be used for ryegrass stocking considering this parameter.

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