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Leaf tissue flows in ryegrass managed under different stocking rates

Mônique Foggiato da Silva^{1*}, Marta Gomes da Rocha², Luciana Pötter², Maria José de Oliveira Sichonany¹, Laila Arruda Ribeiro¹ and Anelise Pereira Hundertmarck¹

¹Programa de Pós-graduação em Zootecnia, Universidade Federal de Santa Maria, Av. Roraima, 1000, 97105-900, Santa Maria, Rio Grande do Sul, Brazil. ²Centro de Ciências Rurais, Departamento de Zootecnia, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil. *Author for correspondence. E-mail: monique_zootecnia@hotmail.com

ABSTRACT. Morphogenetic, structural variables and leaf biomass flows of Italian ryegrass (*Lolium multiflorum* Lam.) were evaluated under two stocking rates: 'Low' and 'High'. These rates were determined by heifers exclusively on pasture or on pasture and supplemented with corn grain. The experimental design was completely randomized following a repeated measure arrangement, two stocking rates, two and four replications of area for the stocking rates 'low' and 'high', respectively. The morphogenetic variables, the number of green leaves and tiller density were similar in both stocking rates. Leaf senescence rate was higher with low stocking rate. Heifers grazed with similar intensity and frequency in both stocking rates. The increase by 33.6% in the stocking rate caused by the use of supplement does not change the leaf biomass flow of Italian ryegrass, but alters its potential efficiency of use near the reproductive stage of the plant.

Keywords: forage intake, frequency of defoliation, intensity of defoliation, morphogenesis, supplementation.

Fluxos de tecidos foliares de azevém manejado sob diferentes taxas de lotação

RESUMO. Foram avaliadas as variáveis morfogênicas, estruturais e os fluxos de tecidos foliares de azevém (*Lolium multiflorum* Lam.) sob duas taxas de lotação: 'Baixa' e 'Alta'. Essas taxas foram determinadas por bezerras, exclusivamente em pastejo ou em pastejo e recebendo grão de milho como suplemento. O delineamento experimental foi inteiramente casualizado com medidas repetidas no tempo com duas taxas de lotação, duas e quatro repetições de área para a taxa de lotação 'Baixa' e 'Alta', respectivamente. As variáveis morfogênicas do azevém, o número de folhas verdes e a densidade de perfilhos foram similares em ambas as taxas de lotação. A taxa de senescência foliar foi superior na taxa de lotação 'Baixa'. As bezerras pastejaram com intensidade e frequência similar em ambas as taxas de lotação. O aumento de 33,6% na taxa de lotação ocasionado pelo uso de suplemento para bezerras em pastejo não altera os fluxos de biomassa do azevém, mas altera a sua eficiência potencial de utilização próximo ao estádio reprodutivo da planta.

Palavras-chave: consumo de forragem, frequência de desfolhação, intensidade de desfolhação, morfogênese, suplementação.

Introduction

Concentrated supplements can be offered to beef heifers to increase individual and per area weight gain, characterizing addition and substitution in the consumption of forage and supplement (Farias et al., 2012). The effects of feeding supplements to grazing animals, on temperate forages, include the 20% increase in stocking rate (El-Memari Neto et al., 2003; Pötter et al., 2010).

Changes in the stocking rate, caused by the supply of supplement, influence directly the intensity and frequency of defoliation of grasses and this can modify the productive and structural variables of the pasture, leading to changes in pasture-animal interactions.

Morphogenetic variables can be used to know the pasture-animal relationship and make decisions

related to pasture management. These variables, along with environmental variables, interfere with growth rates and development of grasses. Monitoring the characteristics of leaf tissue flows dynamics enables to describe the pastoral environment resulting from the changes caused by the action of the herbivorous. Elucidation of these characteristics is especially important when there is an increase in stocking rate due to the use of supplements, since their use is growing in pastoral systems.

Considering the importance of Italian ryegrass (*Lolium multiflorum* Lam.) in livestock production systems in southern Brazil it is, probably, the most studied species in this region. Studies on morphogenesis and leaf tissue flows have already been performed in this forage (Cauduro et al., 2007; Confortin et al., 2010; Confortin et al., 2013; Pontes

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et al., 2004; Pontes et al., 2003), but they do not explain directly, the impacts caused by the increase in stocking rate, as a result of supplementary energy, on the flows of leaf tissue in ryegrass.

This study was conducted to characterize the morphogenetic response and to assess the dynamics of leaf tissue flows in ryegrass grazed by beef heifers under different stocking rates caused by the supply of supplements to grazing animals.

Material and methods

This experiment was carried out from July to September 2010, at the Department of Animal Science, Federal University of Santa Maria (UFSM), located in the Central Depression of Rio Grande do Sul State, Brazil. The climate in the region is humid subtropical (Cfa), according to the Köppen classification. Climatological data for the experimental period were obtained from the Metereological Station of UFSM.

Soil of the experimental área is classified as Paleuldalf (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2006),. The chemical characteristics of the soil at 0-10 cm depth are: pH-H₂O: 5.3; pH-SMP: 6.2; % clay: 24.0 m V⁻¹; P: 10.4 mg dm⁻³; K: 96.6 mg dm⁻³; % OM: 2.4 m V; Al³⁺: 0.3 cm cmolc L⁻¹; Ca²⁺: 3.4 cm cmolc dm⁻³; Mg: 2.0 cm cmolc dm⁻³; base saturation: 64.3%; Al saturation: 4.5%.

A 4.9 hectare experimental area divided into six paddocks, constituting the experimental units, was used. The ryegrass pasture (*Lolium multiflorum* Lam.) was established in April 2010. Fertilization consisted of 250 kg ha⁻¹ of the formula 5-20-20 (NPK) and urea (85 kg ha⁻¹ nitrogen), as topdressing, divided into three applications (27/5, 3/8 and 9/9/2010).

The experimental animals were 44 Angus heifers, with an initial age of eight months and body weight (BW) of 143±10.6 kg. The flows of ryegrass leaf tissues were evaluated under two stocking rates: 'low' (700 kg BW ha⁻¹) - heifers exclusively on pasture and 'high' (1,100 kg BW ha⁻¹) - heifers on ryegrass pasture and daily supplemented with corn grain in a proportion of 1% BW. The grazing method was put-and-take stocking to maintain 1,200 to 1,500 kg ha⁻¹ DM of forage mass.

The stocking rate (SR; kg ha⁻¹ BW) per evaluation period, was calculated by measuring the sum the mean live weights of tester heifers plus the average weight of each heifer used for adjustments in the stocking rate, multiplied by the number of days in the experimental unit, and divided by the number of days in the trial period.

Thermal sum (TS) of each period was calculated by the equation: $TS = S \text{ (tmd -}5^{\circ}C)$, in which tmd

is the daily mean temperature of the evaluation period and 5°C is the baseline temperature for growth of cold season forage species.

The forage mass (FM) was evaluated by the direct visual estimation method with double sampling. Forage from the cuts was homogenized for manual separation of botanical and structural components. After the separation and drying of the structural components in a forced air oven at 55°C for 72 hours, we determined the proportion of leaf blades and stems in the FM. From the proportion of leaves and stems, we determined the leaf/stem ratio (LSR). The forage accumulation rate (FAR; kg DM ha⁻¹) was assessed using three grazing exclusion cages per area repetition.

Forage allowance (FA; kg DM 100 kg⁻¹ BW) was calculated by the equation: ((FM number of days⁻¹) + FAR)/SR of the period. The green leaf blades allowance (LBA; kg DM 100 kg⁻¹ BW) was calculated by: (((FM *% leaves

and calculation of leaf tissue flows, we used the marked tiller technique (Carrere et al., 1997), with measurements in 40 tillers per paddock. The measurements were performed on 16 occasions in the following periods: A- July 13 to 29; B- August 3 to 19; C- August 31 to September) number of days⁻¹) + FAR*% leaves)/SR.

For morphogenetic measures 17. On these occasions, we measured the height of the pseudostem (cm), the extended tiller (cm) and the canopy (cm). The depth of leaf blades was calculated by the difference between average canopy height and the pseudostem height.

The length (cm) and the number of fully expanded and expanding leaves, and their condition (in senescence or not, intact or grazed) were also evaluated. Fully expanded leaves were measured from the ligule to the top, and expanding leaves, from the ligule of the last fully expanded leaf. For senescent leaves, we measured the length of the green portion of the blade.

Leaf appearance rate (number of leaves tiller⁻¹ day⁻¹) was determined by dividing the number of leaves that have appeared in the evaluation period by the number of days in the period. Phyllochron was determined by simple linear regression between the change in the initial and final number of leaves and the accumulated thermal sum of each period. Phyllochron is the inverse of the slope of this regression, expressed in degree-days (°DD).

For the determination of the duration of leaf expansion (°DD), we multiplied the expanding leaves by the phyllochron of the period. The number of green leaves per tiller (GLT) was considered as the number of leaves that were not

senescing, including grazed leaves. The life span of leaves (°DD) was determined by the product of the phyllochron of the period and the number of green leaves of the tiller.

The leaf expansion rate (TxE; cm °C⁻¹) and the senescence rate (TxS; cm °C⁻¹) were calculated by the ratio between the average elongation or senescence of the tiller between two consecutive assessments and the accumulated thermal sum in the same period.

For calculating the defoliation intensity (INT; proportion of the length removed), we identified the grazed leaf blades in each period and used the formula: INT = [(initial length - final length)/initial length]. The frequency of defoliation (FREQ; days of return to the same leaf) was calculated by the formula: FREQ = Number of contacts in the grazing days/(number of possible contacts* evaluation length).

Tiller density (DPP; tillers m⁻²) was determined by counting the ryegrass tillers in four predetermined areas and four random areas of 0.0625 m² each. In the random areas, tillers were cut, subsequently counted, weighed and oven dried at 55°C for 72 hours and weighed again.

Leaf tissue flows, net balance, real efficiency of grazing and the potential efficiency of use were calculated according to Pontes et al. (2004). To determine the consumption, in %BW, we multiplied 100 kg BW by the value of the average consumption flow per treatment and per period and divided by the stocking rate of the treatment per period.

A completely randomized experimental design was used with repeated measures over time, with two SR, and two and four replications of area for treatments 'low' and 'high', respectively. The variables were analyzed using the MIXED procedure of SAS (2004). Selection test structure was performed using the Bayesian information criterion (BIC) to determine the model that best represents the data. The interaction between treatment and periods was broken down when significant at 5% level. Whenever detected differences, the means were compared by *Ismeans*, with 10% significance level.

Results and discussion

The average temperature of the experimental period was similar to the historical average. Rainfall was 63.7 (92) and 59.4% (91.3 mm) higher in July and September, respectively, and 25.6% (28 mm) lower in August. Insolation was 31.5% (42 hours) higher in July and similar to historical averages in August and September.

Stocking rates (SR; p = 0.0694) were: 'high'-1,102 kg BW ha⁻¹, when the heifers were supplemented and 'low'- 732 kg BW ha⁻¹ when the heifers were exclusively on pasture. The increase in stocking rate resulting from the use of supplements was 33.6%. This increased density of animals generates an increase in the probability of defoliation of leaf blades (Lemaire et al., 2009).

There was no interaction between stocking rates and periods (p > 0.10) for the forage allowance, leaf blades allowance, leaf/stem ratio and forage accumulation rate.

The forage mass (FM) was maintained, on average, with $1,476.7 \pm 39.0 \text{ kg DM ha}^{-1}$ (p = 0.2146), within the management range considered suitable for ryegrass (Roman et al., 2007). The increase in SR due to the use of supplements was not enough to change the sward structure. The leaf/stem ratio was similar between the stocking rates and periods (p > 0.10), averaging 2.3. The average proportion of leaf blades in the forage mass was 42.7%, while that of stems corresponded to 26.7%. The forage allowance (FA; p = 0.1525) and the forage accumulation rate (FAR; p = 0.4134) were similar between SR, with mean values of 10.9 \pm 0.8 kg DM 100 kg⁻¹ BW and 44.7 \pm 4.4 kg DM ha⁻¹, respectively.

The increase in SR caused a lower leaf blades allowance (LBA; p = 0.0986), with a mean of 4.6 kg DM 100 kg⁻¹ BW, 35.6% lower than the LBA of the 'low' stocking rate, with 7.1 kg DM 100 kg⁻¹ BW. This variable also differed between periods, being 42.8% higher in the first and second periods, with an average of 6.7 kg DM 100 kg⁻¹ BW, compared to the third period (3.8 kg DM 100 kg⁻¹ BW). Leaf blades are the components preferably consumed by herbivores, due to the lower energy expenditure required for its harvesting in relation to the stem (Hendricksen & Minson, 1980), the lower resistance to breakdown by chewing and the shorter retention time in the rumen ,(Minson, 2012).

No interaction was detected between stocking rates and evaluation periods (p > 0.10) for phyllochron, leaf appearance rate, leaf expansion rate, leaf senescence, duration of leaf expansion, leaf life span, tiller density, height canopy, extended tiller height, pseudostem height, leaf blade depth, number of green, expanding, fully expanded and senescent leaves.

The following variables were similar in both stocking rates: phyllochron (163.8 \pm 4.9 °DD; p = 0.5550), leaf appearance rate (LAR; 0.0074 \pm 0.0002 leaves tiller⁻¹ day⁻¹; p = 0.6470) and leaf expansion duration (257.2 \pm 11.2 °DD; p = 0.5110). These characteristics are genetically determined, but

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may be influenced by temperature, nutrient supply, soil moisture and management (Lemaire et al., 2009).

There were differences between the evaluation periods for phyllochron (p <0.0001) and LAR (p <0.0001). The highest value (201.2 °DD) for phyllochron was found in the third period (8/31 to 9/17) of pasture use, reflecting the lower leaf appearance rate (0.0058 leaves tiller day had been decrease in the appearance rate, as new leaves appear, as they run a greater distance between the meristematic apex and the leaf end, requiring increased thermal accumulation for expansion. The leaf expansion duration (p = 0.0001) was intermediate in the first period (259.4 \pm 11.0 °DD), higher in the third (302.2 \pm 11.0 °DD) and lower in the second period (210.1 \pm 11.0 °DD).

Leaf expansion rate (p = 0.2708) was, on average, 0.06 ± 0.003 cm °C⁻¹ and similar in both SR, probably because phyllochron and forage mass were similar too. This is the morphogenetic variable that is, separately, the most closely correlated with the forage mass (Horst et al., 1978).

A significant difference (p = 0.0458) was found for the senescence rate between stocking rates, being higher in paddocks with 'low' SR, with a mean of 0.125 ± 0.007 cm °C⁻¹ and 31.4% lower in paddocks with 'high' SR. This result indicates a better grazing efficiency in the 'high' SR. This efficiency can be defined as the proportion of forage accumulation in the pasture consumed by animals before senescence (Lemaire et al., 2009). Thus, the increase in stocking rate possibly increased the use of forage produced. Along the ryegrass cycle, there was 23.1% reduction in the senescence rate (p < 0.0001) and 37.6% in leaf expansion rate (p < 0.0001). These values might be explained by the stagnant production of leaves when the plant reaches the pre-reproductive stage (second and third periods).

There was no difference in the number of green leaves between different SR (p = 0.3674) and periods (p = 0.3940), with a mean value of 3.9 ± 0.3 green leaves per tiller. This value is similar to that found by Confortin et al. (2010), of 3.7 leaves in ryegrass, which is described as a relatively constant genotypic characteristic. The life span of leaves was similar (p = 0.9471) in tillers in both SR, averaging 671.1 \pm 34.5 °DD. Considering the average daily temperature (14.4°C), the lifespan of a leaf was approximately 46.4 days. The fully expansion of a leaf corresponds to the complete senescence of another one (Lemaire et al., 2009) and maintaining a relatively constant number of green leaves in a tiller is due to the balance between the leaf senescence

rate and leaf lifespan. Lifespan of leaves was different between periods (p <0.0001); intermediate in the first period, with an average of 635.6 \pm 27.3 °DD, lower in the second, with an average of 528.89 \pm 27.3 °DD and higher in the third period, 848.73 \pm 27.3 °DD.

The increase in SR resulted in no alteration (p > 0.1) in the number of expanding leaves (NEL; 1.6 ± 0.08), expanded leaves (EL; 2.52 ± 0.2) and senescent leaves (SL; 2.2 ± 0.1). These variables differed between periods. NEL was intermediate in the first period (1.6) and higher in the second (1.7), 9.6% higher than the latter period (1.5; p = 0.0351). EL was 16.1% lower in the first evaluation period (2.3) when compared to the third (2.7), and was not different from the others in the second period (2.5; p = 0.0788). SL was lower in the first period (1.4), intermediate in the second (2.3) and higher in the last period (2.9; p = 0.0002). The production of leaves in a tiller is continuous, the leaves remain green for a certain time (life duration of the leaves) and after this period, the blades that were not removed during the grazing will senesce (Confortin et al., 2010).

There were no differences in tiller density (p =0.1829) between the SR, with an average of 3,705.78 ± 113.5 tillers m⁻². The direct relationship of leaf appearance rate with tiller density determines the tillering potential for a given genotype, because each leaf formed on the stem represents the emergence of a new phytomer, that is, the generation of new axillary buds. The tiller density differed between periods (p < 0.0001), being similar in the first and second periods (3,269.7 ± 141.0 tillers m⁻²) and higher in the last period $(4,577.8 \pm 141.0 \text{ tillers})$ m⁻²). This was not expected for this variable, where the trend is a decrease in tillering with the proximity of the reproductive stage. The observed behavior indicates that the high number of tillers, even at the end of the pasture cycle, is indicative of proper pasture management during its phenological cycle (Barth Neto et al., 2013).

In the two stocking rates, the height of the canopy (9.4 \pm 1.0 cm), the extended tiller (15.0 \pm 0.6 cm), the pseudostem (5.6 \pm 0, 4 cm) and the depth of blades (3.4 \pm 0.6 cm) were similar (p > 0.10). This similarity may be associated with the management to maintain similar forage mass. The depth of leaf blades represented 40.6% of canopy height and is associated with the forage distribution in space that determines the ability of the herbivorous to consume the forage.

Moreover, there was a difference between periods for canopy height (p = 0.0686), which was 1.5 cm lower in the first and second periods (8.9 \pm

0.8 cm) compared with the third one (10.5 \pm 0.8 cm). The extended height of the tiller (p = 0.0220) was 2.2 cm greater in the first (16.6 \pm 0.7 cm) than the second and third periods (14.3 \pm 0.7 cm). Next to the ryegrass flowering, there is the elongation of internodes, increasing the pseudostem height. The pseudostem height (p = 0.0226) was 1.7 cm higher in the last period (6.5 \pm 0.4 cm) compared to previous periods. The pseudostem is a physical barrier for the harvest of leaf blades. The depth of leaf blades (p = 0.0021) was higher in the first period (4.8 \pm 0.5 cm), intermediate in the second $(3.3 \pm 0.5 \text{ cm})$ and lower in the third period $(2.3 \pm 0.5 \text{ cm})$ ± 0.5 cm). The depth of leaf blades is the main factor to determine the individual performance of sheep grazing on ryegrass when there is variation in the value of FM (Roman et al., 2007).

No interaction was found between stocking rates and evaluation periods (p > 0.10) for the flows of growth, senescence and consumption, net balance, real efficiency of use, intake in % BW, defoliation intensity and frequency.

The 'high' SR did not affect the dynamics of biomass flows. The mean values of flows of growth, senescence and intake were 38.2 ± 4.8 (p = 0.8841), 100.9 ± 12.5 (p = 0.2909) and 22.6 ± 2.4 (p = 0.8662) kg DM leaf blades ha⁻¹ day⁻¹, respectively. According to Pompeu et al. (2009), under rotational management, the biomass flows components of Tanzania grass are little affected by different levels of supplementation. The sum of the flows of senescence and intake surpassed the growth flow (Figure 1), resulting in a negative net balance, similar between stocking rates (p = 0.2551) and periods (p = 0.6422), with an average of -81.2 ± 10.4 kg DM blades ha⁻¹ day-1. This result can be related to the proportion of senescence flow, 62.1% higher compared to the growth flow. Parsons et al. (1983) emphasized the need to find a balance between the processes of growth, intake and senescence to achieve control of forage availability.

The different SR (p = 0.2813) and the periods (p = 0.8174) did not alter the consumption of leaf blades in % DM BW, which averaged $2.4 \pm 0.2\%$ BW. The dry matter intake predicted for this category is 2.8% (NRC, 2001). Thus, regardless of stocking rates,

heifers grazed the same amount of leaf blades in relation to their body weight.

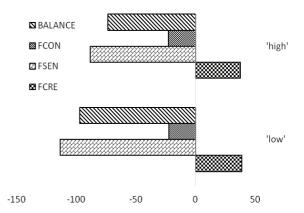


Figure 1. Leaf tissue flows (FCRE = growth flow; FCON = intake flow; FSEN = senescence flow; BALANCE = net balance) in ryegrass under two stocking rates ('high' = 1,102 kg BW ha⁻¹; 'low' = 732 kg BW ha⁻¹).

The intensity (p = 0.8584) and frequency of defoliation (p = 0.3414) were similar in paddocks with different SR. On average, heifers removed $60.9 \pm 3.1\%$ of the ryegrass leaf blade, higher than that reported by Lemaire et al. (2009), around 50% of the length of the leaf blade. This value, according to these authors, remains constant at each defoliation event, independent of the animal density. The return interval of animals to the same leaf averaged 8.6 ± 1.3 days. Probably, the constant forage mass was responsible for the lack of alterations in the defoliation frequency by the animals. Leaf blades were grazed by heifers, on average, 5.2 times until the beginning of senescence.

The real efficiency of use was similar between stocking rates (p = 0.6694) with a mean of 0.61 ± 0.029 , indicating that the growth of leaf blades was higher than the removal of leaf tissue by defoliation, effect allowed by the intensity of removal and the frequency of defoliation.

Likewise, there was no difference in the intake flow (p = 0.5311) and senescence (p = 0.6789) along the grazing cycle, but significant differences (p < 0.1) were detected for the variables growth flow, real efficiency of use, intensity and frequency of defoliation (Table 1).

Table 1. Growth flow, real efficiency of use, intensity and frequency of defoliation of ryegrass in the evaluation periods.

Variables	Periods			D
	A	В	С	— Р
Growth flow ¹	46.65 a (± 5.03)	38.12 ab (± 5.03)	29.97 b (± 5.03)	0.0760
Real efficiency of use	$0.52 \text{ b} (\pm 0.03)$	$0.54 \text{ b} (\pm 0.03)$	$0.77 \text{ a } (\pm 0.03)$	0.0011
Intensity of defoliation ²	$60.38 \text{ a } (\pm 3.81)$	$53.0 \text{ b} (\pm 3.81)$	69.45 a (± 3.81)	0.0370
Frequency of defoliation ³	6.22 c (± 1.58)	13.03 a (± 1.58)	$7.42 \text{ b} (\pm 1.58)$	0.0198

Period A: July 13 to 29; Period B: August 3 to 19; Period C: August 31 to September 17. 1kg DM leaf blades ha⁻¹ day⁻¹; 2% leaf blade; ³days. Values followed by different lowercase letters in the same row are significantly different (p < 0.10) by Ismeans test.

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The growth flow was 55.6% (16.7 kg DM leaf blades ha⁻¹ day⁻¹) higher in the first period compared to the third period, and the second period was not different from the others. The frequency of defoliation was greater in the first period; heifers took 6.8 days less for returning to the same leaf compared to the second period. In this sense, there was a higher growth flow and a higher defoliation frequency in the first period.

There was no difference in the defoliation intensity (64.9%) between the first and the third period. Heifers removed approximately 12% more of the leaf blade during the grazing at the start of pasture use and when the grass cycle approached the reproductive stage, compared to the second period.

The real efficiency of use was 30.8% higher in the third period, in relation to the first two, which were similar. The greater availability of leaf blade at the beginning of the use of pasture and the greater supply of new tissue possibly provided greater harvest efficiency of this material, resulting in greater efficiency of forage use. The highest real efficiency of use at the end of the ryegrass cycle may result from the increased defoliation intensity. In this period, the intensity reached almost 70% of the leaf blade. Real efficiency of use values of less than one for all evaluation periods evidence that the consumption of leaf blades was lower than their growth.

Furthermore, the potential efficiency of use of ryegrass pasture showed interaction between stocking rates and periods (p = 0.0353). In the first (-1.14 \pm 0.5) and second (-1.91 \pm 0.5 kg DM blades ha⁻¹ day⁻¹) periods, the potential efficiency of use was similar between the different SR. In the third period, the potential efficiency of use was 2.1 times higher in paddocks with 'high' SR (-1.50 kg DM blades ha-1 day⁻¹) compared to paddocks with 'low' SR (-3.14 kg DM blades ha⁻¹ day⁻¹). The average negative value indicates that the senescence flow was greater than the growth flow. Excessive loss of plant tissues through senescence necessarily implies low use of accumulated forage. Heifers could potentially have consumed more forage when supplemented due to the replacement of forage by supplement, since supplementation usually increases the total dry matter intake, decreasing, however, forage intake, providing increased stocking rate (Freitas et al., 2005).

Conclusion

For the same forage mass, there is greater efficiency in grazing when the stocking rate is increased by supplementing grazing animals. The

intensity and frequency of defoliation in ryegrass is similar, regardless of the stocking rate.

The higher stocking rate resulting from the use of supplements does not change the leaf tissue flows in ryegrass. Animals consume less forage when receive supplement at the end of the ryegrass cycle.

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