



Methods to determine forage intake by lambs on Italian ryegrass

Aline Tatiane Nunes da Rosa*, Marta Gomes da Rocha, Luciana Pötter, Larissa Arnhold Graminho, Tuani Lopes Bergoli and Paulo Henrique Moterle

Universidade Federal de Santa Maria, Av. Roraima, 1000, 97105-900, Santa Maria, Rio Grande do Sul, Brazil. *Author for correspondende. E-mail: anunesdarosa@yahoo.com.br

ABSTRACT. This study was conducted to evaluate the use of chromium oxide and the content of fecal nitrogen to estimate forage intake by female lambs on Italian ryegrass pasture. The pasture was managed under different forage allowances (FA): 6, 9 and 12% BW. The experiment was a randomized split-split plot design, in which the forage allowances were considered as main plots, the phenological stages as subplots and methods to determine intake as sub-subplots. Forage intake, in the ryegrass vegetative stage, is independent of forage allowance. Lower allowances limit forage intake from the pre-flowering stage, while at the reproductive stage, intake was lower in FA6, intermediate in FA9 and higher in FA12. The estimation of organic matter intake by lambs on Italian ryegrass is similar when chromium oxide and fecal nitrogen are used as markers. The forage intake according to forage allowances depends on the sward structure and is limited by high stem mass at the end of the phenological cycle.

Keywords: *Lolium multiflorum* Lam., fecal nitrogen, lambs, chromium oxide, intermittent grazing.

Métodos para determinação do consumo de forragem por cordeiras em pastagem de azevém

RESUMO. Este experimento foi realizado para mensurar a ingestão de forragem por cordeiras, em pastagem de azevém, utilizando o óxido de cromo e o teor de nitrogênio fecal. A pastagem foi manejada sob distintas ofertas de forragem (OF): 6, 9 e 12% do PC. O delineamento experimental foi inteiramente casualizado, com estrutura de parcelas sub subdivididas, sendo as ofertas de forragem as parcelas principais, os estádios fenológicos, as subparcelas e os métodos de determinação da ingestão as sub sub-parcelas. A ingestão nos estádios vegetativo do azevém independe da oferta de forragem. As menores ofertas limitam a ingestão de forragem a partir do pré-florescimento enquanto no estágio reprodutivo do azevém a ingestão é menor na OF6, intermediária na OF9 e maior OF12. A estimativa da ingestão de matéria orgânica por cordeiras em pastagem de azevém é similar quando são utilizados o óxido de cromo e o nitrogênio fecal como marcadores. A ingestão de forragem em função das ofertas de forragem é dependente da estrutura do dossel e é limitada no final do ciclo do pasto em função da elevada massa de colmos.

Palavras-chave: *Lolium multiflorum* Lam., nitrogênio fecal, ovinos, óxido de cromo, pastejo intermitente.

Introduction

Forage intake is an important factor determining the performance of grazing herbivores through the ingestion of nutrients required for maintenance and production of these animals (Maggioni et al., 2009). The determination of intake in grazing situations, however, has some limitations. In this condition, the intake cannot be determined directly and various techniques are used to estimate it, such as the use of internal and external markers, esophageal fistulas and assessments of feeding behavior. However, each of these techniques has its limitations (Maggioni et al., 2009).

One of the techniques most commonly used to estimate the forage intake is based on the principle that

the fecal excretion is inversely proportional to digestibility, but directly related to the amount of ingested food. Fecal excretion has been more often estimated with the use of external indicators and, among them, the most widely used by the Brazilian scientific community is chromium oxide (Cr_2O_3), despite their known deficiencies (Carvalho et al., 2007; Zeoula et al., 2002). On the other hand, fecal nitrogen has been recently used as a marker for estimates of intake and digestibility of forage (David et al., 2014; Kozloski et al., 2014; Peripolli, Prates, Barcellos & Neto, 2011). According to Peripolli et al. (2011), the use of excretion and fecal nitrogen concentration can promote, with high accuracy, estimates of forage intake and digestibility. Among the advantages of this marker is that there is no need for

dosage of an internal or external marker and did not require sampling of forage as grazed by the animals. This intake can be estimated directly by an equation already determined.

Among the factors that affect the forage intake, the sward structure can be very important, thus the determination of a forage allowance that promotes better structure of the sward and better forage selection opportunity is essential to increase animal productivity in pastoral systems. The use of adequate forage allowance can turn the sheep production system on pastures into a more economical and efficient activity in the winter.

Recent researches evaluated the forage intake of sheep on ryegrass but using the agronomic method (Glienke, Rocha & Confortin, 2008; Roman et al., 2007) and with the double weighing technique (Camargo et al., 2012). These studies did not analyze the relationships with forage allowance nor aimed to determine the better method for the forage intake estimation. This study was conducted in order to compare the use of chromium oxide and fecal nitrogen to estimate forage intake by female sheep on ryegrass pasture under intermittent grazing and managed under different forage allowances: 6, 9 and 12% body weight.

Material and methods

The experiment was conducted at the Federal University of Santa Maria, in the physiographic region called Central Depression, Rio Grande do Sul State, Brazil. The field evaluations were performed from June to October 2012. The soil is classified as Paleudalf (EMBRAPA, 2006) and chemical analysis showed the following results: pH-H₂O: 5.1; pH-SMP: 5.6; % clay: 22.0 mg V⁻¹; P: 7.65 mg L⁻¹; K: 48.0 mg L⁻¹; % organic matter: 2.05 mg V⁻¹; Al³⁺: 0.45 cmol_c L⁻¹; Ca²⁺: 5.1 cmol_c L⁻¹; Mg²⁺: 10.15 cmol_c L⁻¹; effective CEC: 7.8 cmol_c L⁻¹; base saturation: 51.2%; Al saturation: 5.9%. The climate is Cfa, humid subtropical, according to Köppen classification.

The treatments consisted of three forage allowance on ryegrass pasture (*Lolium multiflorum* Lam): 6; 9 and 12 kg dry matter (DM) 100 kg⁻¹ bodyweight (BW) maintained by means of lamb grazing. The experimental area (0.6 ha) was divided into six paddocks totaling two paddocks per treatment, which constituted the experimental units plus a contiguous area of 0.4 ha for the put-and-take animals. The pasture was established in May 2012, with minimum tillage. Fertilization consisted of 250 kg ha⁻¹ of the formula 5-20-20 (NPK) and 100 kg of nitrogen (N) ha⁻¹ in urea form, subdivided into four applications.

The experimental animals were Suffolk female lambs, with age and initial weight of eight months and 43 ± 4.6 kg, respectively. The grazing method was intermittent stocking and grazing interval corresponding to thermal sum (TS) of 250 degree-days, value corresponding to two phyllochron of ryegrass (Confortin et al., 2010). Four occupations were set in each repetition area, which were characterized by the phenological stages of ryegrass: V1: vegetative 1 (6/24-7/7); V2: vegetative 2 (8/7-18); PF: pre-flowering (9/4-15) and F: flowering (10/5-16). The period for pasture occupation plus the interval until the beginning of next occupation was considered a grazing cycle. The intervals lasted 31, 15 and 15 days, respectively, and during these periods, the lambs remained on ryegrass pasture. The first grazing cycle began when the forage mass (FM) reached 1.200 kg ha⁻¹ dry matter (DM).

The forage mass was determined by the following formula: $FM = FM2(i-1) - FM1(i)/D$. Where FM = forage mass of the occupation period, MF1 = pre-grazing forage mass of the grazing cycle "i"; MF2 = post-grazing forage mass of the grazing cycle 'i - 1', D = days of the occupation period. Forage mass was estimated by direct visual estimation technique with double sampling. At the same time, the sward height was measured in cm. From the forage samples of the cuts, we estimated the dry matter content and the botanical and structural composition, by manual separation. All samples were weighed and dried in a forced air circulation oven at 55°C for 72 hours. From this weight, we calculated the percentage contribution and the mass of each component in kg ha⁻¹ DM, for the subsequent calculation of the percentage removal (%) of the components.

The forage accumulation rate was determined by the following equation: $FAR = (FM1(i) - FM2(i-1))/D$. Where: FAR = daily forage accumulation rate; FM1 = pre-grazing forage mass of the grazing cycle "i"; FM2 = post-grazing forage mass of the grazing cycle 'i - 1', D = number of days in the interval between grazing.

Lambs were weighed at the beginning and end of each pasture occupation period, following a six hours fasting from solid and liquid. The average daily gain (kg animal⁻¹ day⁻¹) was obtained from the weight difference between the weighing dates and divided by the number of days of the occupation period.

The stocking rate (kg body weight (BW) ha⁻¹) was calculated by the formula: $SR = (N * (BWt + BWr)/A$. Where: SR = stocking rate; N = number of days that each lamb remained on pasture; BWt = average body weight of the test lambs and BWr =

weight of put-and-take lambs (kg) during the paddock occupation in each phenological stage; A = area of each experimental unit (ha).

The forage allowance was calculated by the formula: $FA = (FM/n + FAR) \cdot 100/SR$. Where FA = forage allowance (%); FM = average forage mass ($\text{kg ha}^{-1} \text{ DM}$) = $[(\text{initial FM} + \text{final FM})/2]$; n = days of the occupation period (days); FAR = daily forage accumulation rate ($\text{kg ha}^{-1} \text{ day}^{-1} \text{ DM}$); SR = stocking rate of the grazing cycle ($\text{kg ha}^{-1} \text{ BW}$). The allowance of green leaves was obtained by multiplying the forage allowance by the average percentage of leaf blades in FM.

Forage intake was estimated, in each pasture occupation period, with the use of chromium oxide (Cr_2O_3). This marker was used in powder form and encapsulated as an indicator of fecal output. Three test lambs per paddock were dosed manually, at 12pm, with 1.0 g Cr_2O_3 per animal. The dosing period was 11 days, with three days for adaptation and eight days for collection of feces directly from the rectum of animals, according to the methodology of Kozloski et al. (2006).

The chromium concentration in dried feces was determined by atomic absorption spectrophotometry (Kozloski, Flores & Martins, 1998). The fecal output was estimated using the formula: $FO = \text{chrome administered (g day}^{-1}) / \text{chromium in feces (g kg}^{-1} \text{ DM)}$, Pond, Ellis, Matis and Deswysen (1989). OM intake (IOM, kg day^{-1}) was calculated by the formula: $\text{IOM} = \text{fecal output} / (1 - \text{forage digestibility})$ and from these data and the stocking rate, it was calculated OM intake as percentage of body weight (IOMBW).

In order to calculate the intake of organic matter of the forage estimated by using the fecal nitrogen (N_{fecal}), it was used the equations proposed by Azevedo et al. (2014) for each phenological stage of ryegrass. Fecal nitrogen (g day^{-1}) was determined by Kjeldahl's method (AOAC, 2005), in the same samples of feces used for the determination of fecal output with chromium oxide. The amount of nitrogen was determined by multiplying this content in feces by fecal output.

From the hand-plucked samples of forage the nitrogen and neutral detergent fiber were determined (AOAC, 2005). All samples were dried in a forced air ventilation oven at 55°C for 72 hours.

Data of pasture variables were analyzed in a completely randomized design with a split-plot arrangement. The forage allowances were the main plots and the phenological stages were the subplots. Paddocks were considered as repetitions. A mixed model was used with the fixed effect of forage allowance, phenological stages and their interactions

and the random effects of the residuals and repetitions nested in the forage allowances evaluated.

The forage intake was analyzed in a completely randomized design, with a split-split-plot structure, the forage allowance as the main plots, the phenological stages as the subplots, and the methods to determine the intake as sub-subplots and the lambs as replicates. A mixed model was used with the fixed effects of forage allowances, the methods to determine the intake, the phenological stages and their interactions and the random effects of the residuals and lambs nested in forage allowances.

After testing normality of data distribution, the variables were analyzed using the MIXED procedure of SAS. We performed a structure selection test using the Bayesian Information Criterion (BIC) that best represented the data. Whenever significant differences were found, mean values of the allowances and phenological stages were compared using the lsmeans option at 10% significance. When there was interaction between allowances and phenological stages, it was broken down when significant at 10%. The variables were subjected to the Pearson correlation analysis and polynomial regression to second order according to levels of forage allowance evaluated.

Results and discussion

The herbage allowances observed were 6.6; 8.8 and 12.6% BW, which are values close to the required allowances of 6, 9 and 12% BW, respectively. There was no interaction between forage allowances \times phenological stages ($p > 0.10$) for the allowance of leaf blades (LBA), but there was a difference for this variable between the evaluated allowances ($p = 0.0195$) and between the phenological stages of ryegrass ($p < 0.0001$). The allowance of leaf blades was higher (3.8) in the allowance of 12% (FA12), intermediate (2.3) in the allowance of 9% (FA9) and lower (1.8% body weight (BW)) in the forage allowance of 6% BW (FA6). The LBA was negatively correlated with the stocking rate (SR; $r = -0.66$; $p = 0.0004$) which, in turn, was negatively and linearly correlated with the forage allowances tested ($SR = 4886.5 - 263.9 FA$; $r^2 = 48.64\%$, $p = 0.0002$; $CV = 27.69\%$). High stocking rates result in reduction in the green leaf blade biomass (Garcia, Carrere, Soussana & Baumont, 2003).

Higher allowances of leaf blades (3.8% BW) were found in vegetative stages 1 and 2. This observation is consistent with the definition of vegetative stage as the moment in which there is greater emergence and expansion of new leaf blades. There was a reduction of 2.5% in the allowance of blades with the advance to the pre-flowering stage and lower LBA was observed at flowering (0.8% BW). Medeiros, Pedroso, Jornada,

Silva and Saibro (2007), however, observed that the average allowance of green leaves (5% BW) was similar between the vegetative, pre-flowering and flowering stages in ryegrass, when forage allowance was 15% BW.

No interaction ($p > 0.10$) was detected between forage allowance \times phenological stages of ryegrass for forage mass and crude protein and neutral detergent fiber in the forage from the grazing simulation. The forage mass (FM; 1207.8 kg DM ha⁻¹; $p = 0.1195$) was similar between the allowances, and probably, this similarity was due to forage accumulation rate (54.3 ± 4.9 kg DM ha⁻¹ day⁻¹) also similar between forage allowances.

The FM differed between stages of ryegrass (Table 1) and was positively correlated with the percentage contribution of stems ($r = 0.78$; $p < 0.0001$), which also differed between the phenological stages of ryegrass ($p < 0.0001$). The percentage contribution of stems increased by 26.9% from the vegetative stage 1 to flowering and this increase, at pre-flowering and flowering in ryegrass, was also accompanied by an increase in forage mass (Table 1). The percentage of stems in the FM differed between forage allowances ($p = 0.0931$), with lower values in FA6 (26.7%), intermediate in FA9 (29.0%) and higher in FA12 (33.0%). The highest percentage of contribution of stems in the FM, as observed in higher forage allowances, may be undesirable because, in this situation, the herbivorous has more difficult to grasp and cut the forage available by increasing the strength needed to harvest the forage similarly to what happens in tropical grasses (Benvenuti, Gordon, & Poppi, 2006).

Table 1. Forage mass (FM, kg DM ha⁻¹), crude protein (CP, %) and neutral detergent fiber (NDF, %) at different phenological stages in ryegrass.

| Items | Phenological stages ¹ | | | | Mean | P* | CV (%) |
|-------|----------------------------------|---------|---------|---------|--------|----------|--------|
| | V1 | V2 | PF | F | | | |
| FM | 936.3c | 1529.7b | 2109.6a | 1851.5a | 1606.8 | 0.0005 | 9.3 |
| CP | 24.0a | 17.7b | 17.7b | 17.7b | 19.3 | 0.0020 | 4.2 |
| NDF | 53.4b | 57.0b | 63.8a | 63.4a | 59.4 | < 0.0001 | 1.5 |

*Values followed by different letters in the same row are significantly different by lsmeans test;

¹Phenological stages: V1: vegetative 1, V2: vegetative 2, PF: pre-flowering; F: flowering.

The forage as grazed showed similar percentages of crude protein (CP; $19.3 \pm 0.8\%$; $p = 0.2427$) and neutral detergent fiber (NDF; $59.4 \pm 0.85\%$; $p = 0.2529$) when the lambs used ryegrass managed with different forage allowances. This characterized a similar diet selection by lambs when subjected to FA ranging from 6 to 12%. The ability of grazing sheep in maintaining the quality of the ingested diet, even under different stocking rates (Garcia et al., 2003) may explain this result, even if the FA6 presented the lowest LBA, which was 107 and 27.5%

lower than the values observed in FA12 and FA9, respectively.

Higher crude protein contents were found in the vegetative stage 1 of ryegrass (Table 1). The crude protein content decreased by 35.5% from this stage to the others, which were similar (Table 1). Changes in the chemical composition of the forage from the vegetative stage, according to Van Soest (1994) are due to reduction in the proportion of leaves in relation to stems and to the beginning of production of seeds by the plant. CP levels verified throughout the ryegrass cycle were not limiting for the development of lambs (NRC, 2007), even if these values have been reduced by 35.6% from the vegetative stage 2 until flowering. Moreover, even the lowest content of NDF (55.2%) observed in vegetative stages 1 and 2 (Table 1) would be considered as limiting values to the intake for grazing animals (Van Soest, 1994).

There was no interaction ($p > 0.10$) between forage allowance \times phenological stages of ryegrass for the percentage removal of forage mass, mass of leaf blades and sward height. The negative linear relationship found between forage allowance and stocking rate led to different removals of forage mass ($p = 0.0632$) and leaf blades ($p = 0.0929$) according to forage allowances. The percentage disappearance of forage mass fitted a negative linear regression model depending on forage allowances (Disappearance of FM = $81.9077 - 4.76FA$; $r^2 = 44.3\%$; $p = 0.0007$; CV = 32.9%), where the reduction of 1% in the forage allowance caused an increase of 4.7% in the removal of FM. Glienke et al. (2010) used different levels of defoliation (removal of 70, 60, 45 and 30% of biomass) in ryegrass pasture used by lambs and observed a reduction of 0.4% in biomass for every 1% increase in the intensity of grazing.

The removal of leaf blades was positively correlated ($r = 0.65$; $p = 0.0010$) with the stocking rate. The greatest removal of blades (74.8%) was registered when pasture was managed under FA6 and intermediate removal (63.7%) in FA9 and the lowest removal were recorded in FA12 (57.8%). The minimum number of animals in FA12 provided a higher percentage of remaining blades and this higher leaf area index could provide a faster recovery in the sward managed under this allowance. On the other hand, it could also be verified a higher senescence rate that would imply greater inefficiency of forage use.

The sward height (SH) fitted a positive linear regression model in terms of forage allowances (SH = $1.3164 + 0.9742FA$; $r^2 = 48.78\%$; $P = 0.0001$; CV = 25.32%). On the other hand, the percentage removal of sward height showed a downward linear trend depending on forage allowances (SH removal = $79.0883 - 4.08FA$; $r^2 = 42.6\%$; $p = 0.0008$; CV =

28.53%) a reduction of 4.1 cm for every 1% increase in the FA. The SH removal showed a negative correlation with the percentage contribution of stems in FM ($r = -0.46$; $p = 0.0264$). The increase in the contribution of stems, depending on the use of greater forage allowances, could have restricted the lowering of the sward height by lambs. These stems may have acted as a physical barrier to defoliation, as a vertical and horizontal barrier to the formation of the bit (Benvenuti et al., 2006).

The similarity in the percentage of forage mass disappearance at different phenological stages (Table 2) was probably due to higher values of this variable with the progression of the pasture cycle (Table 1) and to highest percentage contribution of stems in the FM. This disappearance probably corresponded to the layer of leaf blades present on the grazing horizon. In ryegrass subjected to grazing of sheep, this layer corresponded to 37.1% of the sward height (Roman et al., 2007), a value close to the mean value observed of FM disappearance (Table 2). In addition, the percentage disappearance of FM showed a value very close to the grazing intensity (43%) recommended by Confortin et al. (2010), for a balance between dry matter intake by herbivores and the accumulation of biomass in ryegrass under rotational grazing.

Table 2. Percentage disappearance (%) of forage mass and percentage removal (%) of leaf blades and sward height at different phenological stages of ryegrass.

| Items | Phenological stages ¹ | | | | Mean | P* | CV(%) |
|--------------|----------------------------------|-------|-------|-------|------|--------|-------|
| | V1 | V2 | PF | F | | | |
| Forage mass | 46.7 | 37.7 | 41.2 | 34.6 | 40.0 | 0.4551 | 13.50 |
| Leaf blades | 60.2b | 57.8b | 76.4a | 67.4a | 65.4 | 0.0491 | 7.24 |
| Sward height | 52.0a | 40.7b | 40.3b | 37.9b | 42.7 | 0.0382 | 9.61 |

¹Values followed by different letters in the same row are significantly different by *lsmeans* test; ²Phenological stages: V1: vegetative 1, V2: vegetative 2, PF: pre-flowering; F: flowering.

The removal of leaf blades by ewe lambs (21.9%) was lower in the vegetative stages 1 and 2 when compared to the average removal (71.9%) in the other two stages (Table 2). This variable was negatively correlated with the allowance of blades ($r = -0.62$; $p = 0.0019$), which was reduced with the progression of the pasture cycle. This lower allowance of the component preferred caused the removal of more leaf blades in the stages of pre-flowering and flowering.

In the vegetative stage 1, the sward height removal was 31% higher than the average of the other stages (Table 2) and this variable was negatively correlated with the contribution of stems in FM ($r = -0.46$; $p = 0.0264$). Thus, the reductions observed in sward height were related to the structural changes occurring with the progression of the pasture cycle with increased contribution of stems, senescent material and inflorescences. These changes serve as a barrier to

occur more intense defoliation by lambs. The contribution of stems in the FM ranged from 15% at vegetative 1 to 42% at flowering in ryegrass. It is expected that, with the increased contribution of stems, there is a reduction in the bite size, bite rate, and the forage intake rate (Drescher, Heitkönig, Raats & Prins, 2006).

There was a significant interaction ($p = 0.0390$) for mass of stems (SM), forage allowance \times phenological stages. SM was similar in the different forage allowances in the first three stages of ryegrass, with an average value of 438.97 kg DM ha⁻¹. At flowering, the mass of stems varied positively according to forage allowances (SM = $-202.49 + 110.98FA$; $r^2 = 94.21\%$; $p = 0.0013$; CV = 10.36%). Camargo et al. (2012) observed that in situations of lower intensities of biomass removal (30%), corresponding to forage allowance of 12% BW, there is no stimulus for ryegrass tillering from the vegetative stage, causing an early elongation of internodes. This may explain this accumulation of stems under situations of greater forage allowances.

In turn, no interaction was found ($p > 0.10$) between forage allowance \times phenological stages of ryegrass for estimation of organic matter intake (OMI) of forage determined by chromium oxide and the concentration of fecal nitrogen. The intake values determined by both markers were similar (Table 3) and not different between forage allowances. Thus, both the chromium oxide as fecal nitrogen proved to be suitable for OMI estimates of lambs on pasture. Among the advantages of using chromium oxide as a marker are low cost, easy administration and relative simplicity of analysis required for its determination (Ferreira et al., 2009; Zeoula et al., 2000). These authors report that chromium oxide and titanium dioxide are similar as markers and both can be used to estimate the individual intake of concentrate per lactating cows.

Table 3. Intake of organic matter (OMI) of forage by lambs determined by chromium oxide and fecal nitrogen.

| FA ¹ | OMI Chromium oxide | | OMI Fecal nitrogen | |
|-----------------|-------------------------|--------|-------------------------|--------|
| | kg OM day ⁻¹ | % BW | kg OM day ⁻¹ | % BW |
| FA6 | 0.8231 | 1.65 | 0.7639 | 1.53 |
| FA9 | 0.8455 | 1.69 | 0.7825 | 1.57 |
| FA12 | 0.7783 | 1.57 | 0.7479 | 1.48 |
| CV(%) | 4.34 | 4.07 | 5.45 | 5.30 |
| P* | 0.4264 | 0.4564 | 0.5584 | 0.7479 |
| P** | 0.2501 | | | |

¹Probability of the *lsmeans* test between forage allowances; ²forage allowance; FA6: 6 kg DM 100 kg⁻¹ BW, FA9: 9 kg DM 100 kg⁻¹ BW and FA12: 12 kg DM 100 kg⁻¹ BW;

**Probability of the *lsmeans* test between the indicators chromium oxide and fecal nitrogen.

There was significant interaction ($p < 0.10$; Table 4) between forage allowances \times phenological stages of ryegrass for OM intake (kg day⁻¹). In the

vegetative stages V1 and V2, regardless of forage allowances considered, OM intake was similar (Table 4), averaging 0.69 kg OM day⁻¹. According to Garcia et al. (2003), grazing sheep constantly select green leaves blades in the grazing horizon and this intake similarity may have occurred due to higher allowances of leaf blades and lower stem masses observed at these stages. This allowed the lambs to maintain the preferential harvest of leaf blades (Table 2) allowing, even under management with different forage allowances, a similar opportunity of forage intake.

Table 4. Intake of organic matter (OMI, kg day⁻¹) by lambs in the different phenological stages of ryegrass under different forage allowances (FA).

| FA ¹ | -----Phenological stages ² ----- | | | | | P* | CV(%) |
|-----------------|---|--------|--------|---------|--|--------|-------|
| | V1 | V2 | PF | F | | | |
| FA6 | 0.593a | 0.806a | 0.749b | 0.959a | | 0.0005 | 4.78 |
| FA9 | 0.642a | 0.754a | 0.974a | 0.894ab | | | |
| FA12 | 0.591a | 0.719a | 0.908a | 0.845b | | | |
| Mean | 0.609 | 0.759 | 0.877 | 0.899 | | | |

¹Probability of interaction between forage allowances and phenological stages ($p < 0.10$); ²forage allowance: 6, 9 or 12 kg DM 100 kg⁻¹ body weight (BW); ³phenological stages: V1: vegetative 1, V2: vegetative 2, PF: pre-flowering and F: flowering; *Values followed by different letters in the same column are significantly different by lsmeans test.

In FA6, at the pre-flowering stage, the lambs ingested less 0.19 kg OM day⁻¹ compared to the average intake of organic matter in FA9 and FA12 (0.94 kg OM day⁻¹). In FA6, even if the lambs have removed the highest percentage of forage mass, mass of leaf blades and sward height, this superiority was not enough to keep the OM intake similar to the lambs in FA9 and FA12. The OM intake by lambs was negatively correlated with the mass of stems when these have been managed under FA6 ($r = -0.87$; $p = 0.0040$) and 9% ($r = -0.86$; $p = 0.0055$). For forage mass similar between forage allowances, the increase in the number of lambs to maintain FA6 may have limited the individual intake of forage.

At flowering, the lowest OM intake was found in FA12 (Table 4) without difference from FA9, and this variable was negatively correlated with the mass of stems ($r = -0.63$; $p = 0.0087$). The large accumulation of stems at this stage of ryegrass complicates the selective action of animals (Pedroso et al., 2004). Under situations of lower forage allowances (FA6), it was observed the highest OMI without difference from FA9 and, even at the end of ryegrass grazing cycle, the participation of stems was not enough to restrict the forage intake by lambs.

There was no interaction ($p > 0.10$) between forage allowances \times phenological stages of ryegrass for OM intake as a percentage (%) of the BW and this intake was similar between forage allowances

and stages studied (Table 4), with an average value of 1.64% BW, 44.51% lower than the value predicted by the NRC (2007) for animals in that category. This difference may be because the recommendations of nutritional requirements for sheep, adopted in Brazil, have been developed in other countries and are not always consistent with the performance observed, since the nutritional requirements are influenced by several factors, including environmental conditions, breed (Resende, Silva, Lima & Teixeira, 2008) and management imposed to grazing animals.

Conclusion

The estimated intake of organic matter by lambs on ryegrass pasture is similar when using chromium oxide and fecal nitrogen as markers. Selection of the method to be used depends on the conditions available for analysis and determinations. The consumption of organic matter is dependent on the sward structure resulting from the use of different forage allowances and is limited under the allowance of 12% BW in situations of high mass of stems.

References

- AOAC-Association Official Analytical Chemists. (2005). *Official Methods of Analysis* (18th ed.). Gaithersburg, Maryland, USA: AOAC.
- Azevedo, E., Poli, C., David, D., Amaral, G., Fonseca, L., Carvalho, P., ... Morris, S. (2014). Use of faecal components as markers to estimate intake and digestibility of grazing sheep. *Livestock Science*, 165(7), 42-50.
- Benvenuti, M. A., Gordon, I. J., & Poppi, D. P. (2006). The effect of the density and physical properties of grass stems on the foraging behaviour and instantaneous intake rate by cattle grazing an artificial reproductive tropical sward. *Grass and Forage Science*, 61(3), 272-281.
- Camargo, D., Rocha, M., Silva, J., Glienke, C., Confortin, A., & Machado, J. (2012). Características da ingestão de forragem por cordeiras nos estádios fenológicos da pastagem de azevém. *Arquivos Brasileiro de Medicina Veterinária e Zootecnia*, 64(2), 403-410.
- Carvalho, P. C. F., Kozloski, G. V., Filho, H. M. N. R., Reffatti, M. V., Genro, T. C. M., & Euclides, V. P. B. (2007). Avanços metodológicos na determinação do consumo de ruminantes em pastejo. *Revista Brasileira de Zootecnia*, 36(1), 151-170.
- Confortin, A. C. C., Quadros, F. L. F., Rocha, M. G., Camargo, D. G., Glienke, C. L., & Kuinchtner, B. C. (2010). Morfogênese e estrutura de azevém anual submetido a três intensidades de pastejo. *Acta Scientiarum. Animal Sciences*, 32(4), 385-391.
- David, D., Poli, C., Savian, J., Amaral, G., Azevedo, E., Carvalho, P., & McManus, C. (2014). Faecal index to

- estimate intake and digestibility in grazing sheep. *The Journal of Agricultural Science*, 152(4), 667-674.
- Drescher, M., Heitkönig, I. M. A., Raats, J. G., & Prins, H. H. T. (2006). The role of grass stems as structural foraging deterrents and their effects on the foraging behaviour of cattle. *Applied Animal Behaviour Science*, 101(1-2), 10-26.
- EMBRAPA. (2006). *Sistema brasileiro de classificação de solos* (Vol. 412). Brasília, DF: Embrapa.
- Ferreira, M. A., Valadares Filho, S. C., Inácio, M., Marcondes, M. L. P., Paulino, M. F., & Valadares, R. F. D. (2009). Avaliação de indicadores em estudos com ruminantes: digestibilidade. *Revista Brasileira de Zootecnia*, 38(8), 1568-1573.
- Garcia, F., Carrere, P., Soussana, J., & Baumont, R. (2003). The ability of sheep at different stocking rates to maintain the quality and quantity of their diet during the grazing season. *The Journal of Agricultural Science*, 140(1), 113-124.
- Glienke, C. L., Rocha, M., & Confortin, A. C. C. (2008). Comportamento ingestivo de cordeiras em pastagem consorciada de inverno sob diferentes intensidades de desfolha. *Revista Brasileira de Zootecnia*, 37(11), 1919-1927.
- Glienke, C. L., Rocha, M. G. D., Camargo, D. G., Pötter, L., Confortin, A. C. C., & Costa, V. G. D. (2010). Grazing ecology of female lambs on Italian ryegrass plus red clover pasture under different defoliation intensities. *Revista Brasileira de Zootecnia*, 39(1), 51-60.
- Kozloski, G. V., Flores, E. M. M., & Martins, A. F. (1998). Use of chromium oxide in digestibility studies: variations of the results as a function of the measurement method. *Journal of the Science of Food and Agriculture*, 76(3), 373-376.
- Kozloski, G. V., Maximer, D. P., Oliveira, L., Ivan, A. R., Leite, D. T. L., Maccari, M., ... Sanchez, F. L. F. (2006). Uso de óxido de cromo como indicador da excreção fecal de bovinos em pastejo: variação das estimativas em função do horário de amostragem. *Ciencia Rural*, 36(2), 599-603.
- Kozloski, G., Oliveira, L., Poli, C., Azevedo, E., David, D., Ribeiro Filho, H., & Collet, S. (2014). Faecal nitrogen excretion as an approach to estimate forage intake of wethers. *Journal of Animal Physiology and Animal Nutrition*, 98(4), 659-666.
- Maggioni, D., Marques, J. A., Rotta, P. P., Zawadzki, F., Ito, R. H., & Prado, I. N. (2009). Ingestão de alimentos. *Semina: Ciências Agrárias*, 30(4), 963-974.
- Medeiros, R. B., Pedroso, C. E., Jornada, J. B. J., Silva, M. A., & Saibro, J. C. (2007). Comportamento ingestivo de ovinos no período diurno em pastagem de azevém anual em diferentes estádios fenológicos. *Revista Brasileira de Zootecnia*, 36(1), 198-204.
- NRC. (2007). *Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids* (7th rev. ed.). Washington, DC: Natl. Acad. Press.
- Pedroso, C. E. S., Medeiros, R. B., Silva, M. A., Jornada, J. B., Saibro, J. C., & Teixeira, J. R. F. (2004). Produção de ovinos em gestação e lactação sob pastejo em diferentes estádios fenológicos de Azevém anual. *Revista Brasileira de Zootecnia*, 33(5), 1345-1350.
- Peripolli, V., Prates, Ê. R., Barcellos, J. O. J., & Neto, J. B. (2011). Fecal nitrogen to estimate intake and digestibility in grazing ruminants. *Animal Feed Science and Technology*, 163(2), 170-176.
- Pond, K., Ellis, W., Matis, J., & Deswysen, A. (1989). Passage of chromium-mordanted and rare earth-labeled fiber: time of dosing kinetics. *Journal of Animal Science*, 67(4), 1020-1028.
- Resende, K. T., Silva, H. G. O., Lima, L. D., & Teixeira, I. A. M. A. (2008). Avaliação das exigências nutricionais de pequenos ruminantes pelos sistemas de alimentação recentemente publicados. *Revista Brasileira de Zootecnia*, 37(SPE), 161-177.
- Roman, J., Rocha, M., Pires, C. C., Elejalde, D. A. G., Kloss, M. G., & Oliveira Neto, R. (2007). Comportamento ingestivo e desempenho de ovinos em pastagem de azevém anual (*Lolium multiflorum* Lam.) com diferentes massas de forragem. *Revista Brasileira de Zootecnia*, 36(4), 780-788.
- Van Soest, P. J. (1994). *Nutritional ecology of the ruminant* (Vol. 1). Ithaca, NY, USA: Cornell University Press.
- Zeoula, L. M., Kassies, M. P., Fregadolli, F. L., Prado, I. N., Caldas Neto, S. F., & Dalponte, A. O. (2000). Uso de indicadores na determinação da digestibilidade parcial e total em bovinos. *Acta Scientiarum. Animal Sciences*, 22(3), 771-777.
- Zeoula, L. M., Prado, I. N., Dian, P. H. M., Geron, L. J. V., Caldas Neto, S. F., ... Falcão, A. J. S. (2002). Recuperação fecal de indicadores internos avaliados em ruminantes. *Revista Brasileira de Zootecnia*, 31(4), 1865-1874.

Received on June 6, 2015.

Accepted on August 6, 2015.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.