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Patterns of use of time by heifers with or without supplementation at different phenological stages of winter grasses

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ABSTRACT. A joint data analysis was performed to characterize forage intake rate and patterns of use of time by grazing heifers in cold season pastures. Heifers received or not energetic supplementation at different phenological stages of grasses (vegetative, pre-flowering and flowering). Experiments involved 360 heifers with initial age of eight months and average body weight of 145 ± 17 kg, on continuous grazing method and variable number of animals. Supplemented heifers consumed 17.0% less forage and 22.2% less leaf blades than heifers exclusively on pasture. Grazing activity was concentrated in the afternoon shift, regardless of the feeding system and phenological stages evaluated, with the longest event of rumination during the night. Forage intake rate is similar, regardless of supplement intake and phenological stages of grasses.

Keywords: phenological stages, *Lolium multiflorum* Lam., forage intake rate.

Padrões de uso do tempo por novilhas recebendo ou não suplemento em diferentes estádios fenológicos de gramíneas de inverno

RESUMO. Foi realizada uma análise conjunta de dados, com o objetivo de caracterizar a taxa de ingestão de forragem e os padrões de uso do tempo por novilhas em pastejo de forrageiras de estação fria. Essas novilhas recebiam ou não suplemento energético nos diferentes estádios fenológicos do pasto (vegetativo, pré-florescimento e florescimento). Os experimentos envolveram 360 novilhas, com idade inicial de oito meses e peso corporal médio de 145 ± 17 kg. O método de pastejo foi o de lotação contínua, com número variável de animais. Novilhas recebendo suplemento consumiram 17,0% a menos de forragem e 22,2% a menos de lâminas foliares que as novilhas exclusivamente em pastejo. A atividade de pastejo foi concentrada no turno da tarde, independente do sistema alimentar e dos estádios fenológicos avaliados, e no turno da noite acontece o evento mais longo de ruminação. A taxa de ingestão de forragem é semelhante, independente do consumo de suplemento e dos estádios fenológicos dos pastos.

Palavras-chave: estádios fenológicos, *Lolium multiflorum* Lam., taxa de ingestão de forragem.

Introduction

Adequate nutrition for beef heifers in their first winter is highly relevant so that positive alterations occur in age reduction at their first mating and in birth rates. With these objectives, in south Brazil, these animals have been kept on winter forage pastures (Roso et al., 2009). The provision of energetic supplementation is an alternative to increase the animals' growth velocity in these pastures because the supplements improve the nutrient balance of the diet (Santos et al., 2005).

Herbivores adapt their physiology and feeding behavior to adequately deal with changes in feeding conditions. Physiological responses and anticipation behavior to supplementation are examples of their adaptation (Gregorini, 2012). Supplementation

causes the animals to modify their ingestive behavior (Glienke et al., 2010) through changes in the distribution of behavior patterns during the day (Bremm et al., 2005).

The dynamics and the functioning of pasture ecosystems are influenced by grazing and its components determine the way these animals seek food and process it at different space-time scales (Carvalho, 2013). Short-term forage consumption, ranging from minutes to hours of grazing, is the product of sward structure, accessibility, abundance and quality of forage (Carvalho et al., 2007). The accumulation of stems and dead matter and the reduction of leaf mass occur as the grass phenological cycle advances, with the subsequent decrease in the quality of ingested forage.

Consequently, the animals develop different behavior strategies to adapt themselves to new grazing conditions (Bremm et al., 2005). The ingestion rate is linked to the ability in the forage harvesting by the animal, whilst different grazing managements may cause ruminants to modify the amount of forage ingested per minute (Fonseca et al., 2012). Information of the daily grazing cycles is crucial for the establishment of adequate management strategies.

Knowledge on how ruminants adjust their grazing behavior to cope with changes in canopy structure and the supplement supply is relevant to optimize animal production (Krysl & Hess, 1993). The current assay characterizes the ingestion behavior in hours and ingestion rate of forage by beef heifers grazing on cold season forage species in three phenological stages of grasses (vegetative, pre-flowering and flowering), with or without energetic supplementation.

Material and methods

Data have been retrieved from eight assays conducted between 2003 and 2013 in a 10 ha-experimental area of the Universidade Federal de Santa Maria (UFSM), Santa Maria, Rio Grande do Sul State, Brazil. Ingestive behavior of beef heifers, 8 – 12 months-old, was evaluated. The animals were fed on cold season cultivated pastures, with and without energetic supplementation, and in three grass phenological stages (vegetative, pre-flowering and flowering).

Assays were performed in the physiographic region of the Central Depression of the state of Rio Grande do Sul, Brazil, with humid subtropical Cfa climate, following Köppen's classification. The soil is classified as a Pauleudalf (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2006), with mean values of chemical characteristics of the soil in the experimental area: pH-H₂O: 5.0; pH-SMP: 5.8; clay: 19.2%; P: 13.4 mg L⁻¹; K: 92 mg L⁻¹; MO: 2.7%; Al³⁺: 0.2 cmolc L⁻¹; Ca²⁺: 4.6 cmolc dm⁻³; Mg²⁺: 2.2 cmol L⁻¹; base saturation: 56.6%; saturation Al: 3%.

Three hundred and sixty Angus heifers and Charolais x Nellore crossbreeds, with an initial age of 8 months and body weight of 145 ± 17 kg, were

used. Pastures consisted of black oat (*Avena strigosa* Schreb.), ryegrass (*Lolium multiflorum* Lam.) and red clover (*Trifolium pratense*). Fertilization in the experimental area followed the Chemical and Fertility Soil Commission RS/SC. Mean period for the use of pastures lasted 110 days, between July and November. Meteorological data for May – November were retrieved from the meteorological station of the Universidade Federal de Santa Maria (Table 1).

The grazing method was put-and-take stocking to maintain forage mass (FM) between 1,500 and 2,000 kg of DM ha⁻¹. Two or three area replications were used in all experiments, with three animal-tests per replication. The experimental animals received energetic supplement at a ratio of 0.80% of body weight. Supplementation, provided daily at 14:00, comprised wheat bran, integral rice bran with or without ionophore, oats, corn (whole, laminated and ground), corn ground with crude glycerin and balanced commercial supplement. Grass's phenological stages were divided into vegetative (FM with 0% inflorescences); pre-flowering (FM composed of 4.13% of inflorescences) and flowering (FM composed of 14.13% inflorescences).

Data bank variables of grass and pasture were forage mass (kg DM ha⁻¹), canopy height (cm), mass of leaf laminae (kg DM ha⁻¹), stem mass (kg DM ha⁻¹), mass of dead material (kg DM ha⁻¹), ratio leaf lamina:dead material and stocking rate (kg BW ha⁻¹). Variables of forages harvested as grazed (Euclides, Macedo & Oliveira, 1992) were: crude protein content, *in situ* digestibility of DM and neutral detergent fiber, in DM percentage.

Evaluation of ingestive behavior comprised grazing and rumination time in minutes/hours of the day; forage intake, using chromium oxide as external marker (Rosa et al., 2013); leaf lamina intake (Silva et al., 2015) and forage ingestion rate (g DM min⁻¹) (Benvenuti, Gordon & Poppi, 2006). Ingestive behavior was calculated by visual observations during 18-hour periods.

Most recurring activities were registered on a sheet every 10 minutes (Jamieson & Hodgson, 1979) by three trained observers. Substitution (1) and addition (2) rates of supplement intake on forage intake were calculated by the formulae below (Pötter et al., 2010):

Table 1. Means of temperature, rainfall and insolation, Santa Maria, Rio Grande do Sul State, Brazil (1984-2013).

Items	Months						
	May	June	July	August	September	October	November
Historical means							
Mean temperature (°C)	16.0	12.9	13.5	14.6	16.2	18.8	21.4
Rainfall (mm)	129.1	124.0	145.6	137.4	153.6	145.9	132.2
Insolation (hours)	151.3	125.0	133.1	141.4	160.7	206.8	223.3

Source: INMET (2014).

$$\text{Substitution Rate} = [(\text{IFDMNS}-\text{IFDMS})/\text{IFDMS}]*100 \quad (1)$$

$$\text{Addition Rate} = [(\text{IFDMS}-\text{IFDMNS})/\text{IFDMS}]*100 \quad (2)$$

where: IFDMNS – intake of forage DM by non-supplemented animals; IFDMS – DM intake by supplemented animals.

Variables calculated in the assays were stratified according to the presence or absence of energetic supplementation and phenological stages of the grass (vegetative, pre-flowering and flowering). A graphic analysis of residues was undertaken to verify deviations in linearity. Data were analyzed by a mixed model with the fixed effect of the presence or absence of the supplement, phenological stages and their interactions and randomized effects of the residue and replications in the treatment, with SAS 8.2. Bayesian Information Criterion (BIC) test was applied to select the most adequate co-variance structure for each variable. When differences were detected, means were compared with *lsmeans* procedure at 10% probability.

Results and discussion

Supplemented or non-supplemented heifers were kept on a similar forage mass (FM; 1783 ± 181 kg DM ha⁻¹; $P = 0.1928$) and canopy height (13.7 ± 1.0 cm; $P = 0.9160$). The management produced a similar mass of leaf lamina (578.7 ± 51.6 kg DM ha⁻¹; $P = 0.1380$), stems (481.9 ± 59.2 kg DM ha⁻¹; $P = 0.5939$) and dead material (475.5 ± 74.5 kg DM ha⁻¹; $P = 0.9278$), leaf lamina:stem (1.8 ± 0.3 ; $P = 0.6280$) and leaf lamina:dead material (1.8 ± 0.2 ; $P = 0.4959$) ratio. Forage as grazed provided 22.2% crude protein ($P = 0.7572$), 47.3% neutral

detergent fiber (NDF; $P = 0.2258$) and *in situ* digestibility of DM 78.9% ($P = 0.5349$) for exclusively grazing heifers and for supplemented heifers.

FM in the vegetative stage comprised 45.4% of leaves; 23.4% of stems and 17.9% of dead material (Table 2). When compared to the flowering stage, the participation of leaf lamina in FM was 85.1% greater, stem mass and dead material mass were respectively 1.9 and 2.9 fold smaller. Ratio between leaf lamina:stem and leaf lamina:dead material were respectively 6 and 5.2 times greater in the vegetative stage. The mass of leaf lamina, stems and dead material, leaf lamina:stem and leaf lamina:dead material ratios showed intermediate values in the pre-flowering stage when compared to other phenological stages under analysis.

Canopy height (11.7 cm) and *in situ* digestibility of DM (82.0 % DM; Table 2) were similar in the vegetative and pre-flowering stages. Canopy height in these stages was 3.6 cm (30.5%) less than that in the flowering stage, whereas digestibility was 9.5% higher. Forage as grazed in the vegetative stage had a 9.6% higher crude protein content and 14.0% lower NDF content when compared to that of the flowering stage. In the pre-flowering stage, the contents of crude protein and NDF were intermediate to the others phenological stages.

There was interaction between feeding systems $\times$ evaluation hours for grazing time ($p = 0.0046$; Figure 1A). Grazing time of heifers without supplementation was longer (in minutes) at 7:00 (8.5 min.), 10:00 (6.0 min.), 14:00 (10.8 min.), 16:00 (8.3 min.) and 24:00h (8.1 min.).

Table 2. Structural parameters of cold-season forages in different phenological stages, grazed by heifers.

Variables	Phenological stages			P*	NO**
	Vegetative	Pre-flowering	Flowering		
Forage mass ¹	1521.2 c (± 174.1)	1672.4 b (± 175.9)	2189.8 a (± 175.4)	<0.0001	278
Mass of leaf lamina ¹	690.6 a (± 60.4)	550.7 b (± 64.1)	373.1 c (± 63.4)	<0.0001	278
Mass of stems ¹	356.6 c (± 46.7)	513.9 b (± 51.7)	696.7 a (± 50.6)	<0.0001	278
Mass of dead material ¹	272.8 c (± 72.6)	447.1 b (± 76.3)	803.8 a (± 73.1)	<0.0001	278
Canopy height ²	11.8 b (± 1.5)	11.5 b (± 1.6)	15.2 a (± 1.5)	<0.0001	278
Leaf:stem ratio	2.9 a (± 0.2)	1.3 b (± 0.3)	0.5 c (± 0.06)	<0.0001	278
Leaf:dead material ratio	3.2 a (± 0.3)	1.6 b (± 0.3)	0.6 c (± 0.02)	<0.0001	278
Crude protein ³	25.1 a (± 1.2)	23.9 b (± 1.3)	15.5 c (± 1.3)	<0.0001	278
Neutral Detergent Fiber ³	42.4 c (± 2.2)	46.9 b (± 2.2)	56.4 a (± 2.2)	<0.0001	278
In situ digestibility of DM ³	83.2 a (± 5.3)	80.8 a (± 5.3)	72.5 b (± 5.3)	<0.0001	278

Means followed by different letters on the same line are different ($p < 0.05$) by *lsmeans*. *P: probability; **NO: number of observations; ¹kg DM ha⁻¹; ²cm; ³% DM.

Source: Author.

Grazing time was similar for supplemented and non-supplemented heifers at the other hours analyzed. Regardless of the feeding system, the highest animal concentration rate in grazing occurred at dawn (7:00h), with 56.5% of the heifers and between 16:00 and 19:00h, with 59.0% of the heifers.

In the afternoon occurred the longest grazing event (166.2 min.) for heifers receiving supplement or not and this is featured by Gregorini (2012) as the most intense grazing event. It occurred regardless of time spent for supplement intake on supplement heifers. Total grazing time by heifers without supplementation was 56.7 minutes longer than that of heifers with supplementation (324.4 minutes; $p < 0.0001$). Evening grazing was 30.6% of total grazing time by exclusively grazing heifers and for those with supplementation. This value lies within the 25 – 48% range in relation to total grazing time (Penning, Rook & Orr, 1991). The above behavior pattern is linked to fluctuations in the chemical composition of forage, with increase in non-structural carbohydrate concentrations and decrease in NDF concentration in the leaves during the evening period (Gregorini, Tamminga & Gunter, 2006). According to Gregorini (2012), concentration in grazing activity at the end of the evening constitutes the most efficient plant use by the animal. Decrease in the grazing time at 14:00h by supplemented heifers is related to the supply of supplement and may be due to the behavioral adaptation when the daily supplement is received. The animals are deft in adapting their feeding behavior to copy with management changes (Gregorini, 2012).

There was no interaction between feeding systems $\times$ evaluation hours for the rumination period ($P =$

0.3963). Regardless of the feeding system, heifers mainly ruminated (Figure 1B) during late evening and night period (19:00 - 24:00h), with a decreasing of this activity during the evening (16:00 - 18:00h).

Supplemented heifers consumed 2.94kg DM 100 kg^{-1} BW and 2.53kg DM of leaf laminas 100 kg^{-1} BW. The heifers decreased forage intake by 17.0% (0.42kg DM 100 kg^{-1} BW; $P = 0.0188$) and leaf laminas intake by 22.2% (0.46kg DM 100 kg^{-1} BW; $P = 0.0406$) when compared to intake by heifers exclusively on grazing. Total intake of dry matter was 12.24% (0.41kg DM 100 kg^{-1} BW; $P = 0.0219$) higher for supplemented heifers, or rather, an addition rate of 56% and a replacement of 0.44kg of forage DM per kg of ingested supplement. Positive associated effects are reported in high quality pasture when there is an excess of nitrogen derived from forage and when energy supplementation is provided to the animals (Doyle, Francis & Stockdale, 2005).

Lowest forage intake by supplemented heifers may have been due to the physical replacement of one feed by another (Rosa et al., 2013) since the replacement effect of feed may have caused ruminal physical limitation (Doyle et al., 2005). Heifers averagely consumed 1.56 kg DM supplement day^{-1} , corresponding to 24% of total DM daily ingested by the animals.

Forage intake by exclusively grazing heifers and by those with supplementation was 84.1% composed of leaf laminas. Regardless of the feeding system, the intake value demonstrates that the management of the forage mass between 1,500 and 2,000 kg DM ha^{-1} favors the selection of grazeable forage by heifers.

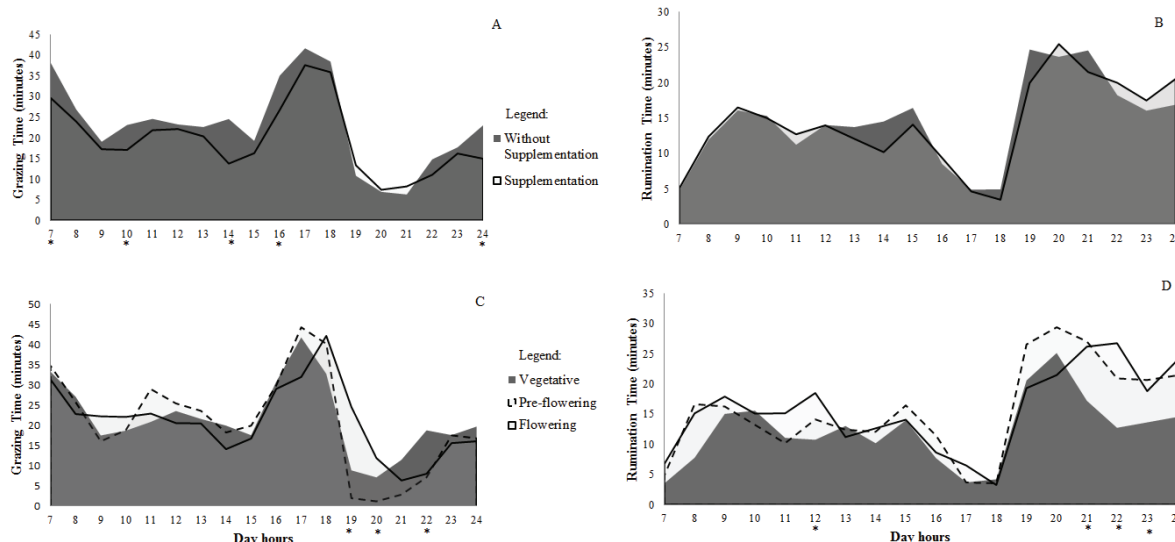


Figure 1. Hourly distribution of grazing activity (A; C) and rumination (B; D) of beef heifers in winter forage pastures with or without supplementation and in different phenological stages of grass (Vegetative, Pre-flowering and Flowering; * means differ at the evaluation timetable by *lsmeans*.)

Source: Author.

The intake of leaf laminas in the two feeding system showed a negative correlation with stem mass

($r = -0.40$; $P = 0.0011$). Canopy stems are a barrier which makes difficult the apprehension of the leaf laminas (Benvenuti, Gordon & Poppi, 2008).

Forage intake was similar ($2.47 \text{ kg DM } 100 \text{ kg}^{-1} \text{ BW}$) in the pre-flowering and flowering stages, or rather, 22.7% less when compared to the vegetative one ($3.03 \text{ kg DM } 100 \text{ kg}^{-1} \text{ BW}$; $P = 0.0029$). Forage intake by heifers at flowering-stage is associated negatively to the stem mass ($r = -0.51$; $P = 0.0087$) associated with NDF content ($r = 0.32$; $P < 0.0001$). Forage intake by the animal is associated to its capacity in filling the rumen and the digesta passage rate. Since the stems have more lignified cell walls, they remain a longer time in the rumen, a fact which may limit forage ingestion (Decruyenaere, Buldgen & Stilmant, 2009).

Forage intake rate ($16.8 \text{ g DM minute}^{-1}$) was similar in feeding systems and in the grass's phenological stages. Similarity in ingestion rate by supplemented and non-supplemented grazing heifers may be due to the fact that this variable is mainly associated to canopy height (Mezzalana et al., 2014), similar in the evaluated feeding systems. According to Gregorini et al. (2009), canopy with greater accessibility in leaf laminas make the ruminants select forage in a shorter time. Results underscore that an increase in forage mass and canopy height due to the pasture's phenological progress may be an adequate management strategy so that the heifers maintain unchanged forage ingestion rate. Ingestion rate lies between 15.8 and $34.0 \text{ g DM minute}^{-1}$, reported in the literature (Gibb, 2006; Chapman et al., 2007).

There was interaction between the grasses's phenological stages $\times$ evaluation hours for grazing time ($p < 0.0001$) and rumination ($p = 0.0001$; Figure 1 C and D). In the pre-flowering stage, there was a 22.6 min decrease in grazing time at 19:00h when compared with the flowering stage and a 8.3 min. decrease at 20:00h when compared to the others. In the vegetative stage, grazing time at 22:00h is 11.2 min higher than in the other phenological stages. Grazing time was similar in the other periods under analysis within the phenological stages. Regardless of the phenological stage, 60% of heifers graze between 16:00 and 18:00h (Figure 1C). In the morning the animals are motivated to graze due to hunger internal stimuli, whereas evening grazing is related to higher nutritional value of the forage (Gregorini, 2012). Distribution of the animals' daily grazing activity (Figure 1A and C) confirms that

night grazing represent a small portion of daily total grazing time (19.0%) and this grazing event is shorter (73.2 min.) and less intense, with only a slight contribution in the intake of daily forage (Gregorini, 2012).

Changes in the canopy structure of the vegetative stage for flowering (Table 2) determine changes in the behavior of heifers throughout the grazing hours (Figure 1C and D). The concentration of grazing activity in the evening in the flowering stage started at 15:00h and grazing lasted till 21:00h. The strategy probably reflects the animal's attempt to guarantee during the day the necessary forage amount to attend to its nutrient requirements. According to Medeiros, Pedroso, Jornada, Silva and Saibro (2007), the grazing ruminant is subjected to several factors that may influence forage ingestion. The opportunity of the animal in selecting its diet may be one of the factors, since selective grazing compensates the low forage quality by the intake of the most nutritive parts of the plant. Selective grazing, however, increases total grazing time (Rutter, Orr, Penning, Yarrow & Champion, 2002). In the evening, the animals' bite mass have more nutrients than those in other periods of the day. Grazing in the evening is highly favorable when ingested/spent nutrient proportions are taken into account (Baggio et al., 2008). The above behavior may be due to an increase in temperature and insolation during the flowering stage (Table 1) which stimulates the animals for late evening grazing to satisfy their daily nutritional needs.

Regardless of the phenological stages under analysis, supplemented or non-supplemented heifers demonstrate a circadian rumination pattern, mostly during the night (Figure 1 B and D), with a preference to the 19:00 - 24:00h period (Gregorini et al., 2012). Parsons, Newman, Penning, Harvey and Orr (1994) also emphasize that highest forage ingestion rate at the end of the evening occurs due to use of the night period for rumination. In fact, this period increases predation risks and the animals increase their state of vigilance (Gregorini, 2012).

At 12:00h, in the flowering stage, the heifers increased by 7.7 min rumination time when compared to that in the vegetative stage, with rumination time in the pre-flowering stage similar to the other stages analyzed. Heifers' rumination time during the vegetative stage was less (in min.) at 21:00 (9.4 min.), 22:00 (11.1 min.), 23:00 (6.1 min.) and 24:00 (8.2 min.) when compared to the other phenological stages. This variable was similar in all the stages analyzed at all the other hours (Figure 1D). Rumination is usually longer during the night

even though rumination periods are subjected to the rhythms of feed supply during the day, which is flexible and follow the grazing behavior of the animals (Gregorini et al., 2012).

Conclusion

Heifers may maintain their forage intake rate regardless or not of energy supplementation or changes in the canopy structure in the grasses's phenological stages. Supply of the energetic supplement to heifers grazing on cold season forages causes modification in their pattern of daily grazing, with a lower grazing duration at 7:00, 10:00, 14:00, 16:00 and 24:00h when compared to exclusively grazing heifers. Changes in canopy structure according to the phenological stages of the grasses cause the heifers to modify their daily rumination pattern.

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