



## Restricting access time at pasture and time of grazing allocation for Holstein dairy cows: Ingestive behaviour, dry matter intake and milk production



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### ABSTRACT

The objective of this study was to assess the effects of restricting access time to pasture and time of grazing allocation on grazing behaviour, daily dry matter intake (DMI), rumen fermentation, milk production and composition in dairy cows. Twenty-one autumn-calving Holstein cows were assigned to one of the following 3 treatments: providing access to a daily strip of pasture for either 8 h between 07:00 and 15:00 h (T7–15), 4 h between 07:00 and 11:00 h (T7–11), or 4 h between 11:00 and 15:00 h (T11–15). The experimental period consisted of 3 weeks of adaptation and 6 weeks of measurements. Cows were offered a daily herbage allowance of 18 kg DM/cow to ground level, 6.1 kg DM/day of a ground sorghum grain-based supplement and 5.2 kg DM/day of maize silage. Milk yield was greater for cows with 8 h access time to the pasture (25.4 vs. 24.1 for 8 and 4 h access time, respectively). Milk yield was not different between cows that access early (T7–11) or late (T11–15) to the grazing session. Milk protein yield was greater for cows with 8 h access time (0.75 kg/d) vs. 4 h access time treatments (0.72 kg/d). Cows with late access time to grazing in the morning produce more protein (0.74 kg/d) than cows with early access to the pasture (0.70 kg/d). Duration of access had a significant effect on herbage DMI (8.3 vs. 6.6 kg/d, for 8 and 4 h access, respectively), but there was no significant effect of time of grazing allocation. Intakes of concentrate and maize silage DM did not differ between treatments.

Pasture depletion rate was significantly slower when cows had access to the pasture for 8 h compared with 4 h (0.04 vs. 0.09 cm/h), but was not affected by allocation time in the 4-h treatments.

Cows grazed for significantly longer in treatment T7–11 than T11–15, achieved significantly more biting and non-biting grazing jaw movements. However, because herbage DMI did not differ between treatments T7–11 and T11–15, it appears that cows grazed more efficiency on treatment T11–15.

The present study showed that reducing the period of access to pasture from 8 to 4 h decreases DMI and milk production. Cows that started their 4-h grazing session later in the

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morning (T11–15) produced more protein than cows that started earlier (T7–11), probably as a consequence of a larger bite mass and a tendency for higher intake rate. Rumen pH of cows grazing on treatment T11–15 declined faster than in cows on T7–11, which is in accordance with the higher VFA and ammonia rumen concentrations observed after the grazing session started.

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## 1. Introduction

Milk production is greatly (70%) determined by dry matter intake (DMI), and to a lesser degree by efficiency digestion of the diet (Chilibroste et al., 2005). In dairy production systems, where cows are confined and fed total mixed rations, the quantity and quality of nutrients offered can be controlled, and adjustment of the diet to cows' requirements to optimize milk production has been studied extensively (Bargo et al., 2003). In contrast, in grazing systems, pasture DMI cannot be easily estimated (Smit et al., 2005) and prediction of milk production is often unreliable. Moreover, under grazing conditions, DMI is frequently insufficient to meet dairy cows' genetic potential for milk production (Kolver and Muller, 1998). In pasture-based systems where herbage allowance becomes restrictive, cows may be supplemented with silage and/or concentrates. However, determining the effects of interaction between plants, animal, and supplements on DM intake and productive performance that has been scarcely addressed (Chilibroste et al., 2007).

Dry matter intake on grazed pastures is mainly determined by herbage mass (DM or OM kg/ha), herbage allowance and duration of access, and by pasture characteristics such as sward height, density and botanical composition (Chilibroste et al., 2005). Such factors constrain bite mass (BM, mg DM/bite) and bite rate (BR, bites/min) which together determine short-term intake rate (IR, g DM/min). At the same time, the actions of searching and selection by the animal compete with biting within grazing time (GT), which with IR determine total daily DMI (Newman et al., 1994). However, grazing management may modify the daily pattern of grazing, rumination, and idling times (Gibb et al., 1998; Orr et al., 1997). The manner in which these patterns are combined modify the supply of nutrients and their utilization (Gibb et al., 1997).

Although IR in grazing dairy cows has been studied comprehensively, most studies are based on short-term observations (e.g., duration of 1 h or less; Hodgson, 1985). Besides the sward characteristics mentioned above, IR is affected by animal characteristics (physiological status, nutritional requirements and appetite) and management (Gibb, 2006; Patterson et al., 1998). The effect of restricting the period of access to pasture on grazing behaviour, daily DMI and productive performance of dairy cows is poorly understood. Restricting the period during which cattle have access to pastures can increase herbage production and utilization by reducing the negative effects of cattle on the sward, such as treading and fouling. Studies with beef cattle, in which the period of access to pasture has been restricted, have shown variable effects on grazing behaviour and performance, depending upon the severity of access restriction and the grazing conditions (Gekara et al., 2005; Smith et al., 2006).

A recent study of dairy cows (Perez-Ramirez et al., 2008) showed that restricting access to pasture from 8 to 4 h per day decreased GT by 2 h which, despite large increases in IR and proportion of available time spent grazing, reduced herbage intake and milk production. As far as we are aware, few studies (Chilibroste et al., 2007) with dairy cows have addressed the effect of restricting access time at pasture, whilst maintaining supplementation (silage+concentrates) at a fixed level, on ingestive behaviour, milk production and composition.

The hypothesis of this study was that the effect of restricting access time at pasture from 8 to 4 h on grazing behaviour, DMI and productive performance, would depend on timing of grazing allocation during the day. The objective of this study was to evaluate the effects of differing durations of access to pasture and time of grazing allocation on daily grazing pattern and behaviour, DMI, rumen fermentation and milk production and composition, in dairy cows.

## 2. Materials and methods

### 2.1. Experimental design, animals, and treatments

The experiment was carried out between May 21 and July 20, in the autumn/winter period, with 3 weeks of adaptation (wk -3, -2 and -1) and 6 weeks of measurements (wk 1–6). It was conducted at the Experimental Research Station "Dr. M.A. Cassinoni" (EEMAC) of the School of Agronomy (Paysandú, Uruguay, 32°S, 58°W), on a 2nd year mixed pasture containing 35% *Trifolium repens*, 15% *Lotus corniculatus*, 35% *Festuca arundinacea* and 15% weeds (DM basis). Animal procedures were approved by the Animal Experimentation Committee of Experimental Station.

Twenty-one autumn-calving Holstein cows yielding  $25.3 \pm 2.53$  kg milk/day, at  $60 \pm 10.3$  days in milk and  $550 \pm 48.8$  kg live weight (LW) were blocked by parity, milk yield and days in milk and randomly assigned to one of three treatments in a randomized block design. Treatments consisted of cows having access to a daily strip of pasture for 8 h between 07:00 and 15:00 h (T7–15), or for 4 h either between 07:00 and 11:00 h (T7–11), or between 11:00 and 15:00 h (T11–15).

During the pre-experimental period, cows were offered the same feeds as in the experiment; with 6 h access to the pasture.

During the experimental period, the area of the daily strips was determined by measurement of the pre-grazing herbage mass to ground level (DM kg/ha), and adjustment to provide a daily herbage allowance of 18 kg DM/cow. In addition, at each milking cows were individually offered 3.5 kg/day of a supplement consisting of a mixture (80:20

as-fed basis) of a commercial ground sorghum grain-based concentrate and whole cottonseed, and after the afternoon milking, 5.2 kg DM/day of maize silage (Table 1). Weights of concentrate and maize silage offered and refused were recorded daily to determine feed intake. Concentrate and maize silage samples were collected from feed-troughs during three consecutive days in week 2, 4, and 6, dried at 60 °C, and stored for subsequent analysis to determine chemical composition.

Cows were milked twice daily (05:30 and 15:30 h) and milk yield was recorded. Milk samples at each milking on two consecutive days per week were collected to determine milk fat, protein, and lactose composition with a MilkoScan (Foss Electric<sup>®</sup>, 133b-Rajasthan, India). Cow LW was recorded on week 2, 4, and 6.

## 2.2. Herbage mass and pasture depletion

To determine the appropriate paddock sizes, herbage mass was calculated weekly using a double sampling technique adapted from Haydock and Shaw (1975). Every 14 days, three replicate sets of five sampling locations were selected within the areas to be grazed. The five locations were chosen to represent a short, a tall and three areas of intermediate sward height. At each location, sward height was measured to the nearest 0.5 cm using a rising plate metre (RPM, Ashgrove Co., Palmerston North, NZ) and 30 × 30 cm squares of pasture on the same area were cut to ground level with shearing scissors. The

cut herbage was collected, weighed, and sampled for determination of DM content to calculate herbage DM mass and derive a linear regression relating sward height (RPM). Each week, herbage mass was calculated by measuring sward height with the RPM at 20 points within the paddocks and applying the regression determined the current or previous week.

The temporal pattern of pasture height depletion during grazing was estimated weekly, during weeks 1–6, by measuring sward height with the RPM at 1-h intervals during the grazing session (minimum of 20 points per strip/h).

During weeks 1–4, and 6, samples of pasture (at least 30 samples per strip), representative of the herbage selected by cows, were plucked by hand from un-grazed areas of sward, for chemical analyses (Table 2).

## 2.3. Herbage DM intake

Individual herbage DMI was determined during the last 4 days of measurement period (wk 6) in 12 cows (4 complete blocks). Herbage DMI was determined using n-alkanes (Dove and Mayes, 2006), with n-tritriacontane (n-C33) as an internal marker and n-dotriacontane (n-C32) dosed as external marker. Herbage intake was estimated by subtracting the amount of n-alkanes derived from the supplements (silage and concentrate) according to Dove and Mayes (2006). During the last 12 days of the measurement period (wk 5 and 6) at each milking, cows were dosed with a cellulose bolus containing 323 mg of n-alkane (n-C32); thus every cow received a daily dose of 646 mg/d. Herbage samples representing the forage selected by cows over the final 4 days were collected by hand plucking following the grazing path of individual cows for 10 min every hour during the grazing session. Samples were dried at 60 °C, and stored until analyses to determine the content of n-alkanes (n-C32, n-C33 and n-C35). Faeces were collected from the rectum of each cow after every milking over the final 4 days of the measurement period and stored frozen at –20 °C until analyses.

## 2.4. Grazing behaviour

Grazing, ruminating and idling times, and the number of grazing jaw movements were recorded for four cows on

**Table 1**  
Chemical composition of supplements.

	Maize silage	Concentrate	Cotton seeds
Dry matter (DM, g/kg fresh)	327	870	901
Organic matter (OM, g/kg DM)	952	920	952
Crude protein (g/kg DM)	68	187	232
Neutral detergent fibre (g/kg DM)	486	–	506
Acid detergent fibre (g/kg DM)	273	197	403
Net energy lactation (Mcal/kg DM) <sup>a</sup>	1.47	1.68	1.82

<sup>a</sup> Estimated from equation of National Research Council (2001).

**Table 2**  
Chemical composition of allowed herbage mass by treatments.

	Treatments			SEM	P-value
	T7–15 <sup>a</sup>	T7–11 <sup>b</sup>	T11–15 <sup>c</sup>		
Dry matter (DM, g/kg fresh)	208	228	223	25.4	0.734
Organic matter (OM, g/kg DM)	837	831	859	38.8	0.766
Crude protein (g/kg DM)	195	201	212	34.4	0.888
Neutral detergent fibre (g/kg DM)	366	353	368	14.9	0.559
Acid detergent fibre (g/kg DM)	212	198	208	25.7	0.851

<sup>a</sup> (T7–15) grazing between 07.00 and 15.00 h.

<sup>b</sup> (T7–11) grazing between 07.00 and 11.00 h.

<sup>c</sup> (T11–15) grazing between 11.00 and 15.00 h.

the two 4-h treatments (T7–11 and T11–15) during weeks 2–4, using automatic behaviour recorders (Rutter et al., 1997). The cows studied were those used to measure herbage DMI in week 6. A recorder was fitted to one cow on each treatment after the afternoon milking (16:00 h) and removed the next day before afternoon milking. Twenty-four hours after recorder removal, the procedure was repeated using two different cows, one cow per treatment, in order to obtain 4 complete recordings per treatment over 7 days. The complete procedure was then repeated to obtain another four recordings per treatment. Under our experimental conditions we occasionally failed to complete recordings due to equipment damage or failure, so recordings were repeated in an attempt to obtain eight recordings per treatment. The mean duration of recordings was  $1402 \pm 8.5$  min. Data were analyzed using the software Graze (Rutter, 2000) and inter-meals intervals and grazing bouts were interpreted as defined by Gibb (1998).

### 2.5. Chemical composition

Hand-plucked samples of pastures and samples of feed were analyzed to determine DM, ash, CP, NDF, and ADF content according to AOAC (2000). Hand-plucked samples of herbage collected during the intake determination period were composited by paddock, and the faeces samples dried at 60 °C were composited for each cow before analyses of n-alkane content (Dove and Mayes, 2006). Diet dry matter digestibility was estimated from the mean concentrations of n-C35 according to Dove and Mayes (2006).

### 2.6. Rumen fermentation study

Simultaneously and adjacent to the previous experimental procedures, six rumen-cannulated primiparous lactating cows yielding  $19.5 \pm 4.58$  kg milk/day, at  $68 \pm 7.4$  days in milk and  $448 \pm 19.0$  kg LW, were blocked by milk yield and days in milk and randomly assigned to T7–11 and T11–15 treatments. Cows grazed individually, tethered within a circular plot as described by Chilbroste et al. (2000). The mean plot size was of approximately 115 m<sup>2</sup>/cow/d aimed to achieve an herbage allowance of 18 kg/DM/cow/d. Each time there was a variation in herbage mass the individual plot area was adjusted as appropriate. Herbage mass was measured by the same method as described for the main experiment. Feeding and milking management were also the same for both studies which ran simultaneously during 6 weeks. From wk 1–6, one day per wk, rumen fluid was collected from cows on T7–11 and T11–15 at 0, 1, 2, 4, 8, 10, 11, 12, 14, 18, and 22 h and 0, 1, 2, 4, 6, 7, 8, 10, 14, 18, and 22 h, respectively, (0=beginning of grazing), to determine pH, ammonia and VFA concentrations. Rumen samples were filtered through a cheese cloth and an aliquot sample was taken immediately to measure pH with a mobile pH-metre (Oakton, Eutech Instruments, Malasia). Aliquots samples acidified in ratio of 20:1 relation, with sulphuric (95.6%) and orthophosphoric (85%) acids were collected and frozen until analysis of ammonia and VFA contents, respectively. Ammonia was determined by distillation with MgO

(Bremner, 1960) and VFA by gas-chromatography (Chilbroste et al., 2000).

### 2.7. Calculations and statistical analysis

Net energy for lactation (NE<sub>L</sub>) was calculated as described by National Research Council (2001). Milk energy output was calculated weekly as  $NE_L = \text{milk yield} \times [(0.0929 \times \text{fat}\%) + (0.0563 \times \text{true protein}\%) + (0.0395 \times \text{lactose}\%)]$ , using milk composition data derived weekly from analysis of the four consecutive samples (National Research Council (2001)).

All statistical analyses were conducted with SAS Systems programs package (v. 9.2, SAS Institute Inc., Cary, NC). Milk yield (calculated as weekly means) and composition, and LW were analyzed in a mixed model with repeated measurements in time, using the MIXED procedure with week as the repeated effect and first-order autoregressive as the covariance structure. The Kenward–Rogers procedure was used to adjust the denominator degree of freedom. The model included treatment, week, and the interaction treatment  $\times$  week (when  $P < 0.20$ ) as fixed effects and blocks as random effect. Pretreatment values were used as covariates in their respective data analysis.

DMI and grazing behaviour data were analyzed with a model that included treatment and block as fixed and random effects, respectively, while sward characteristics and chemical composition were analyzed in a model that included only the effect of treatment.

Means values for milk variables and DMI were compared by orthogonal contrasts to determine the effect of access time 8 h (T7–15) vs. 4 h (T7–11 and T11–15) and timing of grazing allocation early (T7–11) vs. late (T11–15).

Within each week, depletion rate of pasture height during grazing sessions was calculated using the following model:  $y = a \exp(-kt)$  where  $a$  is the initial pasture height (before grazing),  $k$  the fractional disappearance rate of the pasture and  $t$  the hour from the beginning of the grazing session. NLIN procedure was used and it converged with  $P > 0.95$ . The estimated parameters  $a$  and  $k$  were compared using the MIXED procedure with a model that included treatment as a fixed effect.

Rumen pH, and concentrations of ammonia and VFA were analyzed using the TPSPLINE procedure of SAS using the penalized least squares method to fit a nonparametric regression model. The differences between treatments were tested in a graphic way with confidence intervals of 95% for the complete curves.

## 3. Results

There were no differences in either the chemical composition of allowed herbage mass (Table 2), or in the herbage mass and sward characteristics between treatments at the beginning of each session (Table 3).

### 3.1. Milk yield and composition, and cow live weight

Milk production and composition data are presented in Table 4. Milk, FCM, fat and protein yields were

**Table 3**  
Pre-grazing herbage mass, sward height and daily herbage allowance (HA).

Variables	Treatments			SEM	P-value
	T7-15 <sup>a</sup>	T7-11 <sup>b</sup>	T11-15 <sup>c</sup>		
Herbage mass (kg DM/ha)	1491	1751	1536	160.8	0.264
Height RPM <sup>d</sup> (cm)	6.5	7.0	6.6	0.83	0.814
Daily HA (kg DM/cow)	20.3	21.4	20.4	2.46	0.890

<sup>a</sup> (T7-15) grazing between 07.00 and 15.00 h.<sup>b</sup> (T7-11) grazing between 07.00 and 11.00 h.<sup>c</sup> (T11-15) grazing between 11.00 and 15.00 h.<sup>d</sup> RPM=rising plate metre.**Table 4**

Milk yield, milk composition and live weight of dairy cows allowed access to pasture for 8 h at 07.00 h (T7-15) or 4 h, commencing at 07.00 h (T7-11) or at 11.00 h (T11-15).

	Treatments			SEM	P-value orthogonal contrasts	
	T7-15 <sup>a</sup>	T7-11 <sup>b</sup>	T11-15 <sup>b</sup>		8 vs. 4	T7-11 vs. T11-15
Milk yield (kg/d)	25.4	23.6	24.6	0.76	0.047	0.189
Fat corrected milk 4% (kg/d)	24.8	22.5	23.3	0.73	0.002	0.285
Fat (%)	3.96	3.71	3.66	0.143	0.028	0.701
Fat yield (kg/d)	0.98	0.88	0.87	0.035	0.001	0.688
Protein (%)	3.03	2.98	2.99	0.051	0.354	0.883
Protein yield (kg/d)	0.75	0.70	0.74	0.017	0.025	0.020
Lactose (%)	4.93	4.86	4.94	0.057	0.546	0.135
Lactose yield (kg/d)	1.23	1.14	1.22	0.038	0.153	0.047
Energy milk output (NEI Mcal/d)	18.2	16.5	17.2	0.51	0.002	0.146
Live weight (kg)	538	536	535	8.0	0.707	0.868

<sup>a</sup> (T7-15) between 07.00 and 15.00 h; 8 h.<sup>b</sup> (T7-11) between 07.00 and 11.00 h or (T11-15) between 11.00 and 15.00 h, 4 h.**Table 5**

Daily dry matter intake of herbage, maize silage and concentrate by dairy cows allowed access to pasture for 8 h at 07.00 h (T7-15) or for 4 h, commencing at 07.00 h (T7-11) or at 11.00 h (T11-15).

	Treatments			SEM	P-value contrasts	
	T7-15 <sup>a</sup>	T7-11 <sup>b</sup>	T11-15 <sup>b</sup>		8 vs. 4	T7-11 vs. T11-15
Dry matter intake (kg)						
Herbage	8.3	6.6	6.7	0.68	0.031	0.901
Maize silage	4.7	4.3	4.7	0.22	0.676	0.192
Concentrate	6.1	6.1	6.1	–	–	–
Total	19.1	17.0	17.2	0.58	0.008	0.797
Total digestible	13.1	11.2	11.5	0.75	0.026	0.667
Dry matter intake (g/kg LW)						
Herbage	14.8	12.0	12.1	1.04	0.024	0.954
Total	34.5	31.0	31.7	1.56	0.056	0.666

<sup>a</sup> (T7-15) between 07.00 and 15.00 h; 8 h.<sup>b</sup> (T7-11) between 07.00 and 11.00 h or (T11-15) between 11.00 and 15.00 h, 4 h.

significantly higher from cows allowed access to pasture for 8 h, compared with those allowed for only 4 h. Compared with access to pasture for 4 h, access for 8 h significantly increased milk fat content, but did not significantly affect protein or lactose content.

Time of access for the two treatment groups allowed access for 4 h had no significant effect on milk composition or on milk, FCM or fat yield. However, cows allowed access later in the day (T11-15) did produce significantly greater yields of protein and lactose.

NE<sub>L</sub> was significantly greater ( $P < 0.01$ ) for cows on allowed 8 h grazing access (T7-15) than cows allowed 4 h access (T7-11); although there was no effect of time of allocation on the 4-h treatments (T7-11 vs. T11-15). Cow LW did not differ between treatments (Table 4).

### 3.2. Dry matter intake and pasture depletion

Daily DM intakes of dietary ingredients are presented in Table 5. Allowing cows access to pasture for 8 h,

compared with 4 h, significantly increased their estimated intake of herbage DMI. However, estimated herbage DMI did not differ between cows allowed access either early or late in the morning. Intakes of maize silage and concentrate DM did not differ significantly between treatments. Daily intakes of total and digestible DM were significantly greater in cows offered 8 h access compared with 4 h access. The same pattern was observed when herbage and total DMI were analyzed relative to cow LW.

Pasture depletion rate was lower (0.04 cm/h,  $P < 0.05$ ) when cows had access to pasture for 8 h (T7–15), compared with 4 h (Fig. 1). Pasture depletion rate was not affected by the time at which cows entered pasture for 4 h.

### 3.3. Grazing behaviour

Results from the behaviour recordings completed on the two 4-h treatments are presented in Table 6. Cows on treatment T7–11 grazed for 36 min longer ( $P < 0.01$ ) than those on T11–15 and performed more bites ( $P < 0.05$ ) and non-biting grazing jaw movements ( $P < 0.05$ ). There was no significant difference between treatments in either the mean bite rate (51 bites/min) or the number of bites per

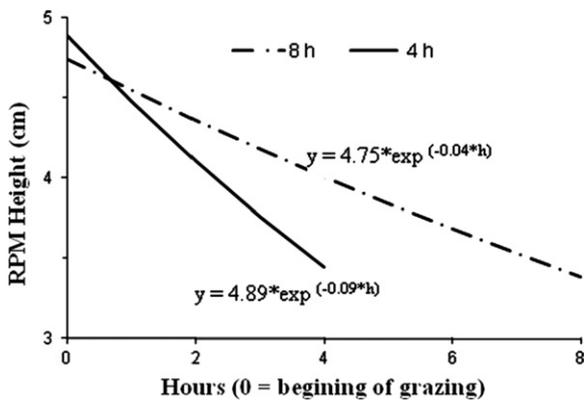


Fig. 1. Regressions representing pasture depletion measured at hourly intervals during grazing sessions lasting 8 h (T7–15, - • -) or 4 h (joint line for T7–11 and T11–15, -).

grazing jaw movement performed by cows. There was no significant effect of treatment on the total time spent ruminating or idling.

From the measurements of herbage DMI, grazing time and grazing bites estimates of short-term herbage intake rates and bite mass were calculated. However, the tentative nature of these estimates, given that grazing behaviour was recorded in weeks 2 to 4 and intakes were measured indirectly in week 6, must be emphasised. The results indicate that significantly greater bite masses were achieved by cows on T11–15 than those on T7–11.

The temporal patterns of grazing and ruminating are presented in Fig. 2. Despite our best efforts, only seven complete recordings were achieved on T7–11. All cows commenced grazing immediately on entering their paddocks and, with one exception on each treatment, showed at least one inter-meal interval. In all cows the majority of ruminating activity occurred during the night and rarely during their time at pasture (Fig. 2). Although there were

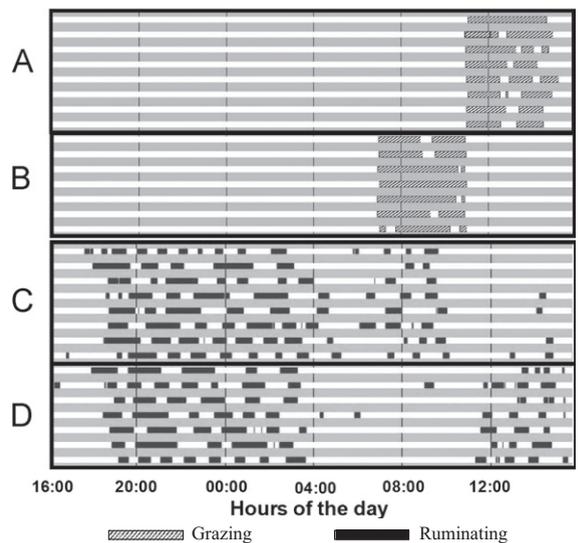
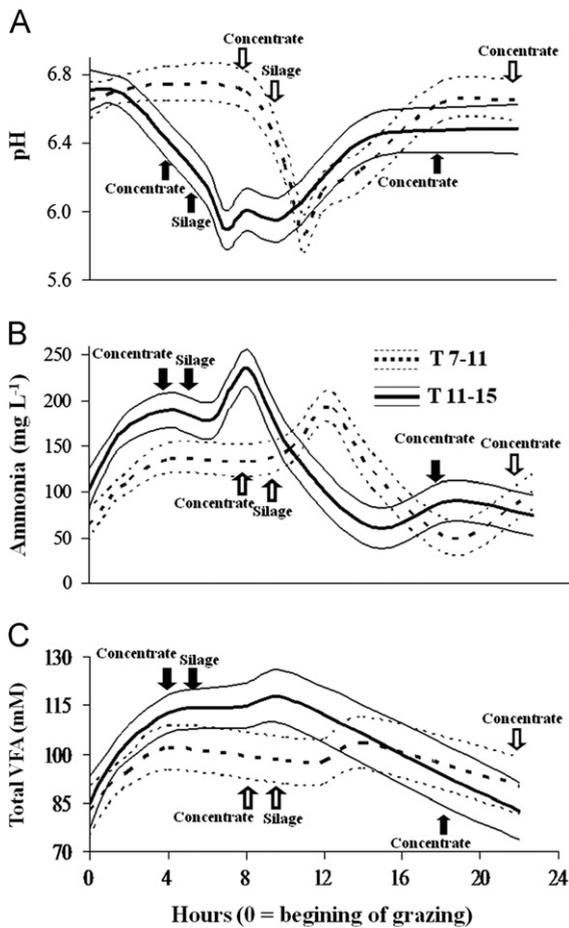


Fig. 2. Temporal patterns of grazing (A, B) and ruminating (C, D) activity by dairy cows allowed access to strips of pasture between 11.00 and 15.00 h (A, C) or between 07.00 and 11.00 h (B, D).

Table 6

Mean time spent grazing, ruminating or idling, number of biting, non-biting and total grazing jaw movements over 24 h, and derived estimates of intake rate and bite mass by dairy cows allowed access to pasture for 4 h, commencing at 07.00 h (T7–11) or at 11.00 h (T11–15).

	Treatments		SEM	P-value
	T7–11	T11–15		
Activity (min)				
Grazing	229	193	9.3	0.002
Ruminating	392	413	11.1	0.362
Idling	665	701	24.2	0.160
Grazing jaw movements (GJM)				
Bites	11,874	9715	925.6	0.038
Non-Biting GJM	5289	3638	648.1	0.028
Total	17,065	13,411	1025.0	0.005
Bites/GJM	0.689	0.730	0.040	0.329
Herbage intake rate (g DM/min)	28.8	36.0	2.79	0.106
Bite mass (mg DM/bite)	594	709	24.2	0.031



**Fig. 3.** Diurnal patterns of ruminal pH (A), ammonia (B) and VFA (C) concentrations of strip-grazed dairy cows with different timing of grazing allocation early in the morning (–T7–11) or late in the morning (–T11–15).

differences in ruminating activity between treatments during the period 07.00–15.00 h, there was little evidence of synchronicity in ruminating activity between or within treatments (Fig. 2C and D).

### 3.4. Rumen fermentation

The temporal patterns of rumen pH, ammonia and VFA concentrations, adjusted to the beginning of the grazing session (0 h), are presented in Fig. 3. Rumen pH was significantly affected by treatment. Cows on T11–15 showed a decline in pH 2 h after the start of grazing, even before the intake of supplements, and reached the minimum value 5 h later (7 h from beginning of grazing session). In contrast, cows on T7–11 exhibited a delay of almost 8 h before pH declined following intake of supplement, achieving the minimum pH value 11 h after the start of the grazing session. Although minimum values of pH did not differ between treatments (pH 5.9), pH remained low for a longer time in T11–15 than in T7–11 cows. After 12 h there were no differences in rumen pH values between treatments and the highest values (pH

6.6) were reached around 16 and 18 h after the beginning of their grazing sessions.

The increase in ammonia concentration following the start of the grazing session was more pronounced ( $P < 0.05$ ) for cows on T11–15 than T7–11; both groups showed a peak concentration approximately 3 h after the start of silage feeding (9 and 13 h after the start of their grazing session, respectively). However, higher peak concentrations of ammonia were recorded in cows on T11–15 than those on T7–11 (232.7 and 196.5 mg/L, respectively).

The temporal patterns of fermentation, as reflected by the total VFA concentrations, were affected by the times of grazing allocation (Fig. 3C). Total VFA concentrations from 6 to 12 h after beginning of the grazing session were greater ( $P < 0.05$ ) in cows on T11–15 than those on T7–11, although no other differences were observed during the remainder of the 24-h period. The VFA molar proportions did not differ between treatments.

Mean daily values for pH (6.4) and total VFA (98.8 mM) did not differ significantly between treatments. However the overall mean daily ammonia concentration was greater for T11–15 than T7–11 (144 vs. 120 mg L<sup>-1</sup>,  $P < 0.01$ ).

## 4. Discussion

Milk yield was 1.3 kg/day (5.1%) less for cows allowed access to pasture for 4 h (T7–11 and T11–15) compared with cows allowed access for 8 h (T7–15). This is largely attributable to the greater herbage DMI achieved on (T7–15) treatment since the concentrate and silage DMI did not differ between treatments. Perez-Ramirez et al. (2008), reported a similar decrease in milk yield (1.1 kg/day and 5%) when the access time to pasture was reduced from 8 to 4 h in groups that started the grazing session at the same time (09.00 h).

Kristensen et al. (2007) working with higher yielding cows found that reducing time at pasture from 9 to 4 h per day, reduced daily milk production from 32.4 to 30.3 kg, equivalent to a 6.5% reduction.

Herbage DMI was 1.7 kg/day lower (19.9%) for cows allowed 4 rather than 8 h access to pasture, resulting in greater milk yield as discussed before. We have found few reports on the effects of restricting access to pasture on herbage DMI by Holstein cows on herbage DMI. Perez-Ramirez et al. (2008) reported a 18.6% reduction in daily herbage DMI (1.9 kg) when duration at pasture was reduced from 8 to 4 h in groups that started the grazing session 09.00 h.

Kristensen et al. (2007) reported a decrease in daily herbage DMI of 2.3 kg (18.1%) when dairy cows were restricted to 4 h compared to 9 h access to pasture. In their study herbage DMI was estimated by the difference between energy requirements for milk production and intake of metabolizable energy (ME) in supplemental feeds, divided by the ME concentration of the hand plucked herbage samples. In the present study cows with a restricted access time to pasture probably increased IR as a behavioural response to the time restriction. However, the potential higher IR of restricted cows did not

fully compensate herbage DMI that was higher for 8 than for 4 h access time (Chilibroste et al., 2007).

Although milk yield was different between cows with 8 vs. 4 h of access time to pasture (T7–15 vs. T7–11 and T11–15), this did not occur for cows that were restricted to 4 h access time to pasture and different timing of grazing allocation early (T7–11) versus late (T11–15). There were several reports that did not find milk yield difference due to timing allocation of the grazing session (Abrahamse et al., 2009 and Kennedy et al., 2009) despite there were difference in access time and herbage management compared with the present experiment.

It is necessary to emphasize that IR was no different ( $P=0.106$ ) from restricted grazing treatments. The cause of non-significance may be due to the lack of power of the experimental design and the low number of cows used to estimate herbage DMI and grazing behaviour, thus we will treat the difference in IR during the discussion as a tendency.

This may be due to the tendency for greater IR and/or more efficient digestive pattern variables that were analyzed in the present study (see discuss below) as were reported previously by Taweel et al. (2004) and Chilibroste et al. (2007).

Pasture depletion rate was slower in 8 than 4 h groups. It can be speculated that cows get adapted to the nutritional management routine, and present different behaviours according to the management. We expected a lower depletion rate of pasture and higher IR for cows that had access to pasture late in the morning (T11–15). Varying the time since the last meal is one of the proposed mechanisms to manipulate feeding motivation (Forbes, 1995). Greenwood and Demment (1988) found that cattle fasted for 36 h grazed 1.5-fold more than those that were not fasted, and that most of the differences could be attributed to a longer initial grazing bout. Similar results were found when time at pasture was reduced from 16 to 8 h in dairy cows during spring: cows of 16 h access time spent 52% of their grazing time compared to 74% of the 8 h access time treatment (Chilibroste et al., 2007). Increased “grazing efficiency” with restricting access time at pasture was reported by Kennedy et al. (2009): cows reduced the proportion of time grazing from 96% to 81% when time at pasture was increased from 6 to 9 h and to 42% with 22 h of access to pasture. Indeed, ruminants learn the rate at which food can be obtained and modify preferences accordingly (Distel et al., 2004). An interesting finding of our study was that independent of the timing of grazing allocation along the day, sward height at the end of the session was not different between treatments ( $3.4 \pm 0.09$  cm). Gibb (2006) described a direct relation between sward surface height and IR, and an indirect relation with grazing time. Several factors mediating ingestive behaviour like residual sward height and density satiety signals and/or fulfillment of requirements could be the cause for the similar sward height found at the end of the grazing session in all groups (Chilibroste et al., 2005).

Grazing behaviour was only determined in the 4 h treatment groups. The reduced grazing time found in T11–15 cows was associated with fewer bites and non-biting grazing jaw movements. However, herbage DMI did not differ significantly between the two treatments, which suggests

that without any significant difference in bite rate, cows on T11–15 were able to achieve a greater bite mass and higher short-term intake rate. Such a proposition is supported by results of other studies. For example Gibb et al. (1998) demonstrated that, under relatively constant sward conditions achieved by variable continuous stocking management, dairy cows increased their bite mass and short-term DM intake rate as the day progressed. Similar results were also reported by Orr et al. (1997). In addition to such increases in short-term intake rate over the course of the day, the chemical composition of the herbage changes, with increases in DM and soluble carbohydrate contents in the afternoon having also been associated with greater herbage DMI later in the day (Orr et al., 2001; Delagarde et al., 2000). This has been interpreted to be an optimum foraging strategy to harvest herbage of higher digestibility, with higher concentrations of soluble carbohydrates and DM (Gibb et al., 1998; Taweel et al., 2004). The tendency for a higher short-term IR in T11–15 cows who spent less time and probably less energy to achieve the same DMI as T7–11 cows, may be related to the greater milk protein yield observed in T11–15 treatment. The IR of T11–15 cows could be caused by the greater fasting time of T11–15 cows as reported before (Chilibroste et al., 2007; Gregorini et al., 2008; Patterson et al., 1998). It is also known that cattle adapt their grazing behaviour in anticipation of future events, including energy requirements, and so can be hyperphagic under certain conditions (Baile and McLaughlin, 1987; Provenza, 1995).

There are several studies associating herbage digestion and rumen fermentation in both confined and grazing animals (Gunter et al., 1997; Van Vuuren, 1993). A majority of recent studies have investigated the relationship between grazing behaviour and rumen fermentation (Chilibroste et al., 2000; Bargo and Muller, 2005; Taweel et al., 2004), whereas very few studies have integrated grazing management, ingestive behaviour, and rumen environment (Chilibroste et al., 2007; Gregorini et al., 2008). Starting the grazing session later during the day (T11–15), produced a faster reduction in rumen pH which is consistent with the increased rumen VFA and ammonia concentrations found in this group. It has been shown that the ingestion of high quality herbage stimulates rapid rumen fermentation, with consequent increase in VFA and ammonia concentrations (Van Soest, 1994). These results were in accordance with the tendency for greater IR observed in cows that grazed later in the morning (T11–15). The greater rumen ammonia concentration in cows on T11–15 may be associated with longer fast time, as shown by Chilibroste et al. (1998), and could result in a minor use of the rumen ammonia. The decrease in ruminal pH recorded after each grazing session has been reported elsewhere, and has a direct relation with grazing session length (Taweel et al., 2004). However, no effect on rumen pH was observed during the first 8 h after the beginning of the grazing session for the T7–11 group. This could be attributed to rumen status at the beginning of the grazing session, determined by the interval since the last meal, to the lower IR and/or a different quality of pasture (less DM and soluble carbohydrates content) eaten by T7–11 cows. We hypothesize that cows that started the grazing session at 11 h (T11–15), with a longer fasting period, had a different rumen status at the beginning of the grazing session, and that this

may also have affected animal grazing attitude (greater appetite) which together with a greater herbage soluble carbohydrate content could have resulted in a more uneven rumen environment and productive performance (greater milk protein yield).

## 5. Conclusions

Restricting access time at pasture from 8 to 4 h decreased DMI and milk production. Within the 4 h treatments, cows that began the grazing session at 11.00 h had a slightly higher IR and produced more milk protein yield than cows that started grazing session earlier in the morning.

The results of this study have a strong practical application as alternative management of the same resources (pastures and animal) may result in an economical benefit. Moreover, a 4 h grazing session starting at 11 h could also be advantageous for pasture care because the sward can be more prone to damage from treading, trampling and fouling because it is wet (i.e. dew moisture) in the early morning, which can lead to increased soil contamination of the pasture.

## Conflict of interest statement

This data has not been published and/or sent to other journal before. The authors have no conflict of interest. Animal procedures were approved by the Animal Experimentation Committee of Universidad de la República (UdelaR, Montevideo, Uruguay).

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