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Effect of restricting time at pasture and concentrate supplementation on herbage intake, grazing behaviour and performance of lactating dairy cows

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ABSTRACT

Restricting time for grazing and concentrate supplementation affects feeding motivation, altering grazing behaviour, and performance of grazing ruminants. This study evaluated the combination of three lengths of restricting time at pasture and two levels of concentrate supplementation on behaviour, intake, and productive performance of dairy cows. Times out of pasture were 0, 4 (0800–1200 h) and 8.5 (0800–1630 h) hours. Levels of concentrate supplementation were 3 and 6 kg DM/cow/day. Measurements were: herbage dry matter intake and digestibility, grazing, ruminating and idling time, bite rate, milk yield and composition, as well as changes in live weight and body condition score. Restricting time at pasture increased ($P<0.01$) grazing time and length of the initial grazing bout ($P<0.01$) and reduced ($P<0.01$) rumination and idling times. Restricting time at pasture did not affect herbage intake or milk yield; however, it reduced milk fat concentration ($P<0.01$). Supplementation level reduced ($P<0.05$) grazing time, but did not affect rumination and idling times. Bite rate was the greatest in cows that were not restricted and had the lowest level in $R_{8.5}S_6$ groups ($P<0.01$). Supplementation reduced herbage dry matter intake, and herbage and total organic matter digestibility ($P<0.01$). Supplementation increased milk yield ($P<0.05$) without effects on milk composition. Modulation of grazing behaviour in response to restricting time at pasture maintained herbage dry matter intake. Changes in grazing behaviour in response to restricting time at pasture plus concentrate supplementation counteract restrictions of restricted time at pasture and thereby help to maintain herbage and energy intake without negative effects on milk production.

1. Introduction

The interaction between the animal' internal state (a.k.a. hunger) and sward characteristics determines foraging behaviour (Pittroff and Soca, 2006) and thereby herbage dry matter intake (HDMI) (Pittroff and Soca, 2006;

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Chilibroste et al., 2007; Gregorini et al., 2009). This interaction can be modulated through feeding practices and, thus, intake pattern aiming to manage HDMI (Chilibroste et al., 2007).

Restricting time at pasture increases the hunger level of cattle (Gregorini et al., 2009). For example, Gregorini et al. (2009) reported increasing levels of ghrelin (an orexogenic hormone) as the restricted time at pasture increased. Restricting time at pasture has been shown to increase short-term HDMI rate, bite mass and bite rate of grazing cattle (Patterson et al., 1998; Perez-Ramirez et al., 2009; Dobos et al., 2009). For example, Patterson et al. (1998) reported increments of 53%, 27% and 22% of HDMI rate, bite mass and bite rate, respectively, during the first meal of the day for grazing dairy cows restricted from pasture for 1–13 h.

Concentrate supplementation often has a negative effect on total daily HDMI and DM intake rate at grazing bout level. Such a negative effect relates to an immediate satiating effect exerted by concentrates (Roche et al., 2007); which reduces the motivation to eat in the next grazing bout after the supplement is fed, thereby reducing eating and searching time while grazing (Krysl and Hess, 1993; Soca, 2006). Despite the reduction in HDMI, concentrate supplementation can be strategically used to either substitute herbage during periods of herbage shortage and/or to enhance total energy intake to increase milk production (Bargo et al., 2003).

There is a substantial body of literature related to foraging ecology and grazing management, as well as research on the effects of supplementation types, management and rates on milk production performance of dairy cattle (Bargo et al., 2003). Previous research studied how grazing time, HDMI and DMI rates are influenced by two sward heights (10 and 13 cm) and grazing durations (1, 2, 4, 8 and 15 h) (Dobos et al., 2009) or changes in moment and time to grazing acces (Kennedy et al., 2011; Mattiauda et al., 2013). However, there is little information regarding how grazing behaviour and milk production/composition are affected by the interaction of both, restricted grazing and supplementation levels, which are among the most frequent changes in feeding of dairy cows (Perez-Ramirez et al., 2009). The restricting grazing time was associated with changes in grazing behaviour and selectivity to meet daily forage intake (Soca, 2006). This behaviour plasticity helps to maintain animal performance and could contribute to reduce metabolic and energetic costs of cows grazing with forage restriction.

We postulated the hypothesis that, during the summer period, restricted time at pasture up to 8.5 h modifies grazing behaviour but not intake or milk production. Therefore the objective of this study was to evaluate the effect of a factorial arrangement of periods of restricted time at pasture and concentrate supplementation levels on HDMI and total intake, grazing behaviour, diet and herbage digestibility and productive response of lactating dairy cows.

2. Materials and methods

2.1. Experimental site and pastures

The study was conducted at the experimental station "Oromo", University of Chile (40°8′S, 73°2′W). Pastures were on the region of volcanic origin (Central Valley Trumao) type soil. The botanical composition of the sward determined by manual separation of forage was *Lolium perenne* (70%), *Dactylis glomerata* (15%), *Anthoxanthum odoratum*, *Bromus sp*., *Trifolium repens*, *and Achillea millefolium* (5%) and other species (10%). There was no change in the ranking of the main pasture species during the experiment.

2.2. Animals, treatments and management

Thirty-six lactating Holstein-Friesian cows (157 ± 10.0 DIM; 550 ± 50.0 kg BW; 2.7 ± 0.5 points BCS, scale 1–5) were grouped by age, DIM, milk production (24 ± 2 l/cow), BW and BCS, and randomly assigned to one of the six treatments (n=6/treatment) based on a factorial arrangement of the following factors:

R=Restricting time at pasture.
- R_0=No restriction – free access to pasture at all times.
- R_4=Restriction period 0800–1200 h (after the morning milking).
- $R_{8.5}$=Restriction period 0800–1630 h (between the morning and the afternoon milking).

S=Level of concentrate supplementation.
- S_3=3 kg DM/cow/day
- S_6=6 kg DM/cow/day

Restricting time at pasture took place on stand-off pads contiguous to the milking parlour. During the restricted time at pasture, cows had unrestricted access to shade and fresh water. Cows were milked at 0700 and 1530 h during approximately 1.5 h. Grazing observation was done during 17:00–23:00 PM, 5:30–7:00 AM and 8:30–14:30 AM–PM.

Concentrate and mineral supplements were individually fed during milking at equal amounts during the morning and the afternoon milking. The concentrate (as DM) consisted of corn grain (60%), sorghum grain (20%), citrus pulp (10%), fishmeal (5%), vitamins and minerals (5%). Concentrate supplement had 11.6 MJ ME/kg DM and 19.5% CP. Cows were rotationally grazed with four days residence time per paddock. Planned herbage allowance was 7.5 kg of herbage DM (ground level) /100 kg BW/ day and kg DM/ha/day respectively. Herbage allowance was set by adjustments of paddock size with an area of 0.66 ± 0.08 ha, during the experimental period. The groups of cows of each treatment grazed in separate pastures.

The duration of the experiment was 95 days, with 10 days of adaptation to the treatments and 85 days of measurements. Before adaptation, the cows grazed similar sward as a single group and supplemented daily with 4 kg of same the supplement used during the experimental period. The experimental design and settings allowed no need for cows to return to previously grazed plots. Treatments were replicated in space and the experimental paddocks were set 200 m apart.

2.3. Measurements

2.3.1. Pasture and supplement

Sward surface height and bulk density were recorded at the beginning and at the end of each grazing period for each paddock in all treatments ($n=200$ per treatment and pasture) using a ruler and a plate metre (Ashgrove Co., Palmerston North, NZ). Herbage mass was determined by a double sampling technique (Haydock and Shaw, 1975). Herbage mass was cut at ground level in $25\times50\ cm^2$ quadrants ($n=25$ samples per treatment and pasture). Samples of cut herbage were oven-dried (60 °C air-forced) for DM analysis and then ground to pass 1 mm screen for further analysis of N (Horwitz et al., 1975) and NDF(Van Soest et al., 1991). During the days of herbage intake determination we employed hand clipping protocol (t'Mannetje and Jones, 2000) to estimate diet quality.

2.3.2. Herbage and supplement intake

Herbage and supplement dry (DM) or organic matter (OM) intakes were determined during 18 consecutive days. Daily concentrate intake was measured for each cow by difference between the amount of supplement offered and refused. Daily herbage OM intake was determined from faecal production and diet digestibility estimates (Coates et al., 2000). All cows were dosed with chromic oxide (Cr_2O_3, Merck®), 12 g/cow/day for 18 days from day 15 to 33 of the experiment. The Cr_2O_3 was administered in pellets mixed with the supplements. Faecal samples were collected from the rectum in each milking during the last 5 days of Cr_2O_3, administration. Daily faecal samples were constituted from the individual milking samples for each animal, oven-dried with (60 °C air forced) and ground to pass 1 mm screen for further Cr concentration determination by atomic absorption spectrophotometry (Perkin-Elmer/373Massachusetts, USA). Faecal production was calculated by relating Cr daily dose and Cr faecal concentration. A recovery of 95% of Cr was assumed.

Diet digestibility was calculated per Eq. 1, using indigestible NDF as internal marker (IICA, 1985):

$$J=[(A+(Y-B)/(A+Y))]100 \qquad (1)$$

where, J is the diet digestibility (%), A is the supplement intake (g DM), Y is the herbage intake (g DM) and B is the faecal production (g DM/day).

Herbage digestibility was determined according to the following equation (IICA, 1985):

$$X=J-(sS/x) \qquad (2)$$

where, X is the herbage digestibility (%), J is the diet digestibility (%), x and s are the percentages of herbage and supplement in the diet, and S is the supplement digestibility (%).

2.3.3. Behavioural activities

Grazing behaviour was recorded for 14 days, from day 19 to 33 of the experiment. Grazing, rumination and idling activities were monitored (Hirata et al., 2002) by trained observers every 5 min from 0700 to 2300 h using 4 distinct focal animals per day per treatment selected for milk production and DIM. Bite rate was determined as time to give 100 prehension bites (Coates, 2000), every 5 min 6 times during the length of the grazing session. The length of the first grazing bout during the available grazing time was determined by the difference between the initial time of such a bout (time of entrance to the pasture break) and its end. This end time was determined by the start time of the first inter grazing bout longer than 5 min (Rook and Huckle, 1997).

2.3.4. Milk yield and composition, live weight and body condition score

Milk yield was determined daily during the experimental period for each cow using individual milk metres. Individual milk samples were taken at each milking for weekly fat and protein concentration analysis. All cows were weighed and evaluated for body condition score (scale 1–5 points; Sniffen and Ferguson, 1991) every 2 weeks. Cows did not have access to water for 12 h before to the weighing.

Milk production was corrected to 4% of fat based on the equation proposed by Tyrrell and Reid (1965): $FCM=MP(0.4+0.15F)$, where FCM is the energy corrected milk (kg/cow/day), MP is the milk production (kg/cow/day) and F is the milk fat percentage (g/kg).

2.3.5. Experimental design and statistical analysis

The study was set as an arrangement of two factors, R and S. Restricting time at pasture had three levels according to its duration (hours), R_0, R_4 and $R_{8.5}$, and S had two levels according to level (kg DM/cow/day) of supplementation, S_3 and S_6. The combinations of factors, treatments, were compared under a completely randomized experimental design (Bransby et al., 2000). For the statistical analysis, sward, milk yield and composition and grazing behaviour were summarised in means per plot used as an experimental unit. The relation between total herbage mass, green and pre and post-grazing sward surface height was analysed by multiple regressions using PROC REG (SAS, 2011).

Behavioural data were expressed as net time and proportion of total time available to graze. Data were transformed by $\sqrt{\text{arcsine}}$ and analysed with a model including R and S levels, day of behavioural observation (D) and the interaction $R\times S\times D$. These data were analysed as repeated measurement in time using PROC MIXED of SAS (SAS Inst., Inc., Cary, NC, USA). The information of grazing behaviour expressed as percentage of total grazing rumia and rest time based arcsine transformed were analysed based on the following model:

$$Y_{ijk}=u+R_i+S_j+\text{Day}_k+(R\text{Day})_{ik}+(S\text{Day})_{jk}+(RS\text{Day})_{ijk}+e_{ijkl}$$

Milk production and composition was analysed as repeated measurements in time using PROC MIXED (SAS) with treatment and Day as fixed effects and cows as the repeated unit. The effect of treatment on production variables (milk and fat production, live weight and body condition score) was tested through regression coefficient heterogeneity and slope heterogeneity analysis, based on the following model:

$$Y_{ijk}=u+R+S+\text{Day}+R\times\text{Day}+S\times\text{Day}+C+e_{iij}$$

where, y_{ijk} is the dependent variable, R is the restricting time at pasture (R_0, no fasting; R_4, fasting between 0800 and 1230 h; $R_{8.5}$, fasting 08:00–1630 h), S=concentrate supplementation (S_3 3 kg DM/cow/day; S_6, 6 kg DM/cow/day), Day is the day since the start of the experiment, C is the covariate, value of dependent variable at the start of the experiment, and e_{iij} is the experimental error.

The PROC MIXED of SAS (SAS, 2011), using the structure of covariance AR (1) was used for this analysis. The $R\times S$ was set as repeated/sub. Within herd variation was used as random effect. Differences between coefficients of slope heterogeneity for $R\times$Day and $S\times$Day were tested using Tukey studentized t-test for LSD with and α protection level of 0.05.

3. Results

3.1. Sward features

Herbage mass and chemical composition and sward surface height did not differ among sets of paddock used for each treatment group. Pre and post-grazing herbage mass averaged 1500 ± 180 (28% DM) and 980 ± 100 kg DM/ha (39% DM), respectively. Chemical composition of herbage offered averaged 116 ± 13; 583 ± 200; 300 ± 100, g/kg DM of CP, NDF, ADF. Pre- and post-grazing sward surface height averaged 6.0 ± 1.6 and 3.5 ± 1.3 cm, respectively. The evolution of pre and post-grazing height was reported in Fig. 1 A.

3.2. Herbage and total organic matter intake and diet digestibility

Restricting time at pasture did not affect herbage and total OM intake (Table 1). On the other hand, increasing S reduced ($P<0.001$) herbage OM intake by 3.9 kg OM/cow/day and tended to affected total intake (Table 1). No interaction among restricting time at pasture and supplementation level was observed for herbage or total OM intake.

Treatment interaction affected the herbage digestibility as increasing levels of concentrates (3 *vs* 6 kg) when cows were not restricted (R_0) or were restricted for 4 h (R_4) decreased herbage OM digestibility, but this did not occurred in $R_{8.5}$ group (Table 1).

Treatment interaction did not affect total OM digestibility (Table 1), but both R and S did ($P<0.001$). Restricting time at pasture increased total diet OM digestibility by 3.0% when comparing R_0 *vs* $R_{8.5}$. The increase in S reduced total OM digestibility by 2.3%.

3.3. Behavioural activities at pasture

In the present study, grazing time as percentage of time at pasture was affected by treatment interactions ($P<0.05$, Table 2). In R_0, the S reduced the grazing time but did not affect it in R_4 and $R_{8.5}$. An increase in grazing activities (expressed as percentage of the total time at pasture) of 29% and 53% was observed when restriction time at pasture was increased to 4 h (R_4) and 8.5 ($R_{8.5}$) respectively. Moreover, the duration (min) of the initial grazing bout was considerably affected by restriction: when compared with R_0, the duration of the initial grazing bout increased by 20 and 140 min ($P<0.001$) for R_4 and $R_{8.5}$, respectively. Thus, even if restricting time at pasture did not affect herbage and total OM intake, it did increase intake rate (R_0=24, R_4=26 *vs* $R_{8.5}$=30 gr DM/min, $P<0.001$).

Rumination and idling (in min) were not affected by nor by R neither by S, but when these variables are expressed as percentage of time at pasture had a considerable effect on rumination and idling times (Table 2). Compared to R_0, R_4 and $R_{8.5}$ reduced rumination time at pasture by 50% and 438%, respectively ($P<0.05$).

The interaction between R and S affected bite rate (Table 2). Bite rate was the greatest for R_0S_3 and R_0S_6 and the lowest for $R_{8.5}S_6$. In the case of the R_0 treatments, S did not have an effect on bite rate; however, R_0S_3 cows showed a greater OM herbage intake than R_0S_6 ($P<0.05$)

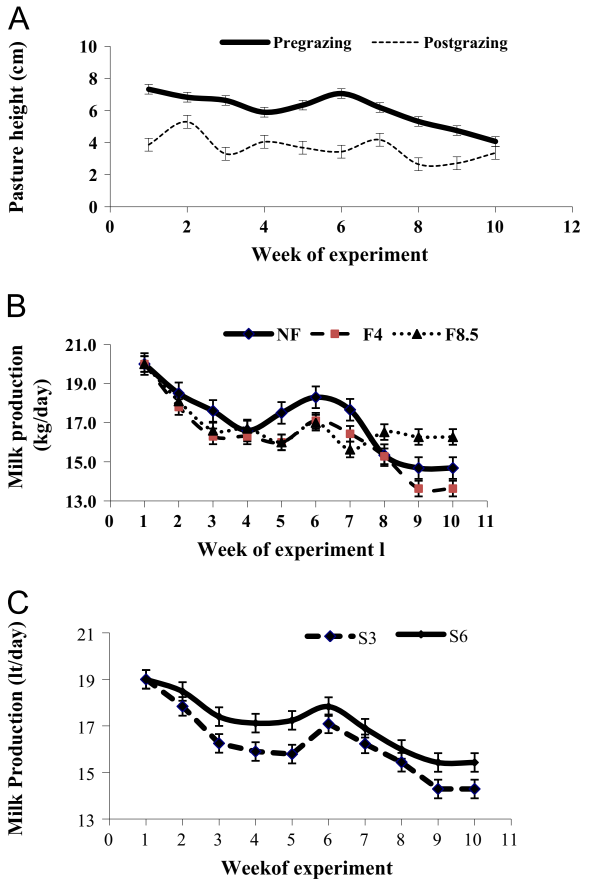

Fig. 1. (A) Evolution of pasture height at pre and post-grazing period (least square means ± standar error). (B) Evolution of milk production in restricting grazing treatment during experimental period (least squares means ± standar error). (C) Evolution of milk production in suplement treatment during experimental period (least squares means ± standar error).

3.4. Milk yield, live weight and body condition

Interaction between $R \times S$ did not affect any productive variable (Table 3). Restricting time at pasture did not affect milk production (Fig. 1B), but reduced fat content and increased protein content of milk.

The S level increased milk production by 0.7 kg/cow/day ($P<0.05$, Table 3 Fig. 1C), without effects on fat or protein content of milk.

Restricting time at pasture reduced the liveweight loss and BCS during the experimental period (Table 3). Compared with R_0, R_4 reduced liveweight loss while $R_{8.5}$ tended to maintain liveweight during the experimental period. Compared with R_0, and R_4, $R_{8.5}$ reduced BCS loss by 0.17 points.

The increment in S slightly reduced liveweight during the experimental period (9.6 kg), without changes in BCS ($P<0.05$).

Table 1
Effect of restricting time at pasture and level of concentrate supplementation on herbage and total OM intake and digestibility of mid lactation grazing dairy cows (Means presented are least square means).

	Treatment						SEM	Effect		
	R_0		R_4		$R_{8.5}$			R	S	$R \times S$
	S_3	S_6	S_3	S_6	S_3	S_6				
Herbage OM intake (kg/cow/day)	14.5	9.7	13.5	9.8	13.8	10.5	2.2	0.64	0.0001	0.39
Total OM intake (kg/cow/day)	17.2	15.3	16.2	15.6	16.5	16.2	0.7	0.74	0.08	0.48
Herbage OM digestibility (%)	59.5	55.4	59.6	55.5	61.8	59.1	1.48	0.0001	0.0001	0.0042
Total OM digestibility (%)	67.5	66.5	67.6	66.6	69.7	69.3	1.6	0.0001	0.0001	0.18

R_0, no restricted time at pasture; R_4, restricting period 0800–1200 h; $R_{8.5}$, restricting period 0800–1630 h; S_3, 3 kg of concentrate supplementation (kg DM/cow/day) and S_6, 6 kg of concentrate supplementation (kg DM/cow/day).

Table 2
Effect of restricting time at pasture and level of concentrate supplementation on behavioural activities of mid lactation grazing dairy cows (means presented are least square means).

	Treatment						SEM	Effect		
	R_0		R_4		$R_{8.5}$			R	S	$R \times S$
	S_3	S_6	S_3	S_6	S_3	S_6				
[a]Grazing time (min/day)	486	438	412	430	355	341	12	0.0001	0.001	0.001
[a]Grazing (%)	67	60	81	83	98	96	0.17	0.0001	0.04	0.02
[b]Rumination (%)	26	31	14	14	0.5	0.8	0.28	0.0001	0.68	0.29
[c]Idling (%)	6.4	8.2	2.2	2	0.3	0.3	0.06	0.0001	0.54	0.12
Duration of the initial grazing bout (min)	84	77	99	102	222	218	0.25	0.00010	0.45	0.42
Biting rate, (bite/min)	55	55	53	49	52	46	5.0	0.01	0.12	0.001

R_0, no restricted time at pasture; R_4, restricting period 0800–1200 h; $R_{8.5}$, restricting period 0800–1630 h; S_3, 3 kg of concentrate supplementation (kg DM/cow/day) and S_6, 6 kg of concentrate supplementation (kg DM/cow/day).

[a,b,c] Mean of 4 cows during 3 days (% of the total time available to graze).

Table 3
Effect of restricting time at pasture and level of concentrate supplementation on milk yield and composition of mid lactation grazing dairy cows (means presented are least square means.)

	Treatment						SEM	Effect		
	R_0		R_4		$R_{8.5}$			R	S	$R \times S$
	S_3	S_6	S_3	S_6	S_3	S_6				
Milk production (kg/cow/day)	16.8	17.4	16.6	16.6	16.1	17.6	1.3	0.3	0.04	0.25
Fat (%)	3.7	3.8	3.2	3.5	2.8	2.9	0.31	0.006	0.8	0.15
Protein (%)	3.2	3.4	3.4	3.3	3.4	3.4	0.06	0.01	0.21	0.1
Energy corrected milk (kg/cow)	16.0	17.0	14.5	15.5	14.3	15	1.0	0.01	0.13	0.30
Liveweight change (gr/day)	−47.0	−20.0	−0.7	−69.0	56.0	−14.3	0.01	0.03	0.02	0.35
Body condition score (pts)	−0.48	−0.21	−0.48	0.25	−0.39	0.05	0.01	0.05	0.05	0.14

R_0, no restricted time at pasture; R_4, restricting period 0800–1200 h; $R_{8.5}$, restricting period 0800–1630 h; S_3, 3 kg of concentrate supplementation (kg DM/cow/day) and S_6, 6 kg of concentrate supplementation (kg DM/cow/day).

The restricting time affected energy corrected milk (R_0=17.0 *vs* R_4=15.0 *vs* $R_{8.5}$=15.5 kg/cow $P<0.01$).

4. Discussion

The restricting time affected grazing behaviour and interacted with the supplement level to affect diet digestibility of milk cows, however it did not affected pasture HDMI. The lack of effect of restricting time at pasture on HDMI is supported by previous results of Gregorini et al. (2009) who did not find reductions in herbage intake by restricting time at pasture (from 8 to 20 h/day) in beef heifers. These results evidence the flexible and compensatory behaviour that the animals use to maintain daily herbage intake.

The reduction in herbage OM intake/kg of supplement in the present experiment was greater than the coefficient of substitution rate for concentrate previously reported (1 *vs* 0.6, Bargo et al., 2003), which can be explained by reduced grazing time, low herbage height and/or digestibility. The lack of effect of the restricted grazing time on total OM intake suggest that the high substitution observed in the present study was due to pasture characteristics as has been reported previously (Berzaghi et al.,1996).

The effect of the concentrate level on herbage digestibility may be related to the proportion of supplement in the diet (S_3=21 *vs* S_6=41%). In S_3 groups, supplement intake was located below the limit known to reduce forage digestibility (Berzaghi et al., 1996). In S_6 group, concentrate was 30% over such a limit (Horn and McCollum, 1987), which could also offer an explanation to the effect of S_6 in reducing herbage digestibility and intake in R_0. This reduction is similar to the results of Berzaghi et al. (1996), who reported reductions in herbage intake and apparent digestibility of the NDF at similar sward conditions, animal features and *S* levels of the present study. Besides, it should be taken into account, rumen environment modifications due to concentrates (reduced rumen pH, increased concentration of propionic acid, reduction in the acetic/propionic ratio and quantity of ammonia) that limit the activity of celulolític bacteria resulting in a reduction of NDF and OM digestibility (Bargo et al., 2003).

The reduction in herbage intake with *S* also can be explained by the change in grazing behaviour (Berzaghi et al., 1996; Bargo et al., 2003). Indeed, the effect of *S* on R_0 afternoon evening decreased grazing minutes (S_3=175 *vs* S_6=140; min; $P<0.001$) and increased rumia time (S_3=170 *vs* S_6=200; min/day; $P<0.001$). Similar patterns have been reported by Sheahan et al. (2011) with grazing dairy cows in New Zealand. These patterns (reduced grazing time and forage digestibility, increased rumia time) contributed to explained the reduction in forage intake with increasing levels of *S* in R_0, but this was not detected in R_4 and $R_{8.5}$. The *S* level interacts with restricting time at pasture to determine the digestibility of herbage intake. The increase in herbage OM digestibility observed in $R_{8.5}$ could be explained by a higher proportion of grazing time during the afternoon grazing sessions, when herbage has a higher content of soluble carbohydrates and a greater digestibility (Griggs et al., 2007). The afternoon grazing session may be associated with improved in diet forage quality via selectivity. This result, was similar to the reported previously by us, in grazing milk cows where fasting and/or timing or grazing moment were manipulated (Mattiauda et al., 2013).

The HDMI estimations suggest that during the experiment, herbage intake was constrained by both, available mass and herbage quality which could have stimulated cows in R_0 to invest more time in searching activities, as reported in dairy cows (Gregorini et al., 2011a) and beef steers (Gregorini et al., 2011b). At the same level of daily herbage intake as cows in R_0, cows in R_4 and $R_{8.5}$ maintained a higher intake rate when grazing. The increase in intake rate worked as a mechanism to compensate for the restriction in time at pasture. The increment in the duration of the first grazing bout as affected by *R* can be related to a potential increment in eating time (grazing= eating+searching). This increment agrees with the results of Gregorini et al. (2009) and Patterson et al. (1998), who reported a hunger dependent eating time for beef heifers and dairy cows, respectively. Indeed, foraging behaviour is altered (via increments in bite mass, bite rate and net grazing time during the main meals) by cattle to compensate for restricting time at pasture and to maintain daily herbage intake (Patterson et al., 1998; Iason et al., 1999; Chilibroste et al., 2007; Pérez-Ramírez et al., 2008). Moreover, *R* could have reinforced the effect of low accessibility by reducing searching time as well (Soca, 2006). Both the treatment differences in grazing time and the *R* effect on the duration of the first grazing bout reflect the greater motivation to graze when cows' time at pasture is reduced, which was translated into no difference in herbage OM intake by treatment. This premise is supported by the results of Gregorini et al. (2009), who reported increasing levels of the orexogenic hormone ghrelin as the length of time restriction at pasture increased.

Moreover, these results may indicate that within the *R* treatments, increments in *S* reduced bite rate, which may be an indication of satiety effect and agree with the results of Chilibroste et al. (2007), Soca (2006) and Gregorini et al. (2009), who reported greater bite rates for hungrier cows and a declining bite rate as the grazing session progressed. This mechanism may have been used as well by cows under the *R* treatments, since at the same or less bite rate and same OM herbage intake with reduced time at pasture, the increments of grazing time as a percentage of time at pasture may have not been enough.

The restricted grazing time reduced percentage of fat and increased protein in milk. The reduction of fat corrected milk (FCM) (R_0=16, R_4=15 *vs* $R_{8.5}$=14 kg FCM/day; $P<0$, 01), was explained by the modification in fat percentage caused by restricted grazing time. Changes in the ingestive behaviour as well as the frequency-sequence of forage- concentrate ingestion could explain both effects. The *S* reduced the forage and total intake, but it did not affect fat and protein milk content. When forage levels in the diet is superior than 50%, benefits of changes in food frequency have not been observed clearly; however, grazing system imposed in $R_{8.5}$, specially in S_6, could be analysed similar to the intake pattern of indoors systems. The foraging strategy employed by R_4 and $R_{8.5}$ to maintain daily herbage intake was mainly to increase intake rate during the time available to graze by increments in grazing time and reductions in rumination and idling time, which disrupted (and or rearranged) the natural sequence (arrangement) of grazing–rumination bouts during a grazing. Time out of pasture (R_4 and $R_{8.5}$) resulted in cows consuming the majority of herbage during the afternoon and/or early in the evening at high intake rates. The consumption of herbage during the afternoon–evening, when it has highest diurnal nutritive value could have led to more glucogenic fermentation patterns in the rumen, and greater microbial protein flow to the duodenum as reported by Gregorini et al. (2012). Moreover, it is known that in the short-term of a meal, high intake rates lead to high rumen fermentation rates and glucogenic patterns of rumen fermentation, reducing ruminal pH, cellulolytic bacteria activity and acetate production (Nocek, 1992). These confirmed that restricting time at pasture was associated to lower rumia during grazing session and jointly with changes and sequences of herbage ingestion could partially explain the reduction of fat and the slight increment in protein content with *R* (Chilibroste et al., 2007). The effect of restricted grazing time on milk composition could result in indirect evidence of the fact that grazing pattern affected glycogenic precursors as propionate and reduced the lipogenic ones as acetate and butyrate.

Supplementation level affected FCM ($S_3=14.4$ *vs* $S_6=15.7$ kg FCM/cow/day; $P<0.001$) and these beneficial effect of the concentrate supplementation have already been summarised (Delaby et al., 2001; Peyraud et al., 2001; Bargo et al., 2003). The response to supplementation (0.3 l/kg concentrate) was lower than the average (0.7 l/kg concentrate) reported in a literature review (Bargo et al., 2003), but are according with experiments employing milk cows in mid lactation (Mallosini et al., 1995; Stockdale, 2007).

The stage lactation may have influenced nutrient partitioning (potentially greater glucogenic supply and reduction in lipogenesis as described previously) in favour of live weight, which occurs naturally in dairy cows during middle and final lactation (Stockdale, 2007). Moreover, this could have been synergised by a reduced energy grazing cost, due to the fact that restricted cows spend more time at the stand-off pad (Chilibroste et al., 2007).

5. Conclusions

Forage intake was not affected by the restricting time which confirms the hypothesis in the present experiment. Concentrate supplementation caused energy substitution and reduction in total consumption. Increasing the level of concentrate depressed the digestibility of the diet and forage, which would explain the reduction in forage intake observed in the higher levels of supplementation in all treatments.

Changes in grazing behaviour counteract restrictions of restricted time at pasture, which achieve the maintenance of herbage and energy intake.

As a result of restricting time at pasture, grazing sessions can be located during times of the day in which herbage has the highest nutritive value and thereby increase diet digestibility, modifying nutrient supply to the host animal and potentially milk composition.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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