Animal, page 1 of 9 © The Animal Consortium 2018
doi:10.1017/S1751731118000794

Effect of timing of corn silage supplementation to Holstein dairy cows given limited daily access to pasture: intake and performance

D. A. Mattiauda[1†], M. J. Gibb[2], M. Carriquiry[3], S. Tamminga[4] and P. Chilibroste[1]

[1]Facultad de Agronomía, Animal Science Department, Universidad de la República, EEMAC, Ruta 3 km 363, Paysandú, CP 60000, Uruguay; [2]Retired, formerly of the Institute of Grassland and Environmental Research, North Wyke, Okehampton, Devon EX20 2SB, UK; [3]Facultad de Agronomía, Animal Science Department, Nutrition Group, Universidad de la República, Garzon 780, Montevideo, Uruguay; [4]Retired, Animal Nutrition Group, Wageningen University & Research, PO Box 338, 6700AH Wageningen, The Netherlands

(Received 10 July 2017; Accepted 12 March 2018)

The timing in which supplements are provided in grazing systems can affect dry matter (DM) intake and productive performance. The objective of this study was to evaluate the effect of timing of corn silage supplementation on ingestive behaviour, DM intake, milk yield and composition in grazing dairy cows. In total, 33 Holstein dairy cows in a randomized block design grazed on a second-year mixed grass–legume pasture from 0900 to 1500 h and received 2.7 kg of a commercial supplement at each milking. Paddock sizes were adjusted to provide a daily herbage allowance of 15 kg DM/cow determined at ground level. The three treatments imposed each provided 3.8 kg DM/day of corn silage offered in a single meal at 0800 h (Treatment AM), equally distributed in two meals 0800 and 1700 h (Treatment AM-PM) or a single meal at 1700 h (Treatment PM). The experiment was carried out during the late autumn and early winter period, with 1 week of adaptation and 6 weeks of measurements. There were no differences between treatments in milk yield, but 4% fat-corrected milk yield tended to be greater in AM-PM than in AM cows, which did not differ from PM (23.7, 25.3 and 24.6 ± 0.84 kg/day for AM, AM-PM and PM, respectively). Fat percentage and yield were greater for AM-PM than for AM cows and intermediate for PM cows (3.89 v. *3.66 ± 0.072% and 1.00* v. *0.92 ± 0.035 kg/day, respectively). Offering corn silage in two meals had an effect on herbage DM intake which was greater for AM-PM than AM cows and was intermediate in PM cows (8.5, 11.0 and 10.3 ± 0.68 kg/day for AM, AM-PM and PM, respectively). During the 6-h period at pasture, the overall proportion of observations on which cows were grazing tended to be different between treatments and a clear grazing pattern along the grazing session (1-h observation period) was identified. During the time at pasture, the proportion of observations during which cows ruminated was positively correlated with the DM intake of corn silage immediately before turn out to pasture. The treatment effects on herbage DM intake did not sufficiently explain differences in productive performance. This suggests that the timing of the corn silage supplementation affected rumen kinetics and likewise the appearance of hunger and satiety signals as indicated by observed changes in temporal patterns of grazing and ruminating activities.*

Keywords: feeding strategy, grazing, grazing pattern, ingestive behaviour, milk production

Implications

The results of this study show that feeding dairy cows with whole-crop corn silage in two meals before and after grazing rather one meal before grazing increase pasture intake and 4% fat-corrected milk (FCM) yield. Results are relevant because they suggest an opportunity to improve cow's performance with the same amount and type of feed on offer.

† E-mail: dma@fagro.edu.uy

Introduction

In housed dairy production systems, where cows are fed total mixed rations, the quantity and quality of nutrients offered can be controlled. Diet formulations to meet cows' requirements, in order to optimize milk production, have been extensively studied (Bargo *et al*., 2003; Hills *et al*., 2015). In contrast, in grazing production systems, in which herbage is the main component of the diet, the interaction between plants, animals, and supplements and its effects on dry matter (DM) intake and productive performance have been less intensively studied (Chilibroste *et al*., 2007).

Moreover, in such situations prediction of cow DM intake (Smit *et al.*, 2005) as well as a nutrient concentration in the diet are uncertain due to the complexity of the grazing process (Chilibroste *et al.*, 2005; Peyraud and Delagarde, 2013).

Herbage allowance may become restrictive due to seasonal variations in forage growth, periods of extreme rainfall, poor management decisions or any combination of the three. Under these circumstances restricting access time to pasture can be used as a management tool to increase grazing efficiency and pasture utilization (Chilibroste *et al.*, 2007, 2015; Gregorini, 2012). Restricting herbage allowance or access time to pasture reduces herbage DM intake and milk production by dairy cows (Chilibroste *et al.*, 2012; Mattiauda *et al.*, 2013), necessitating the provision of supplementary feeds to meet the nutritional requirements of the milking herd (Armstrong *et al.*, 2010; Peyraud and Delagarde, 2013). Dairy cows may be supplemented with silage and concentrates, which are frequently offered separately during the day. Depending upon the composition, amount and when the supplements are offered, these can influence the timing and duration of grazing meals, herbage selection and digestive processes, and as a consequence dairy cow performance (Chilibroste *et al.*, 2008).

Although cows can graze at any time throughout the day, major grazing events generally occur early in the morning (at sunrise), late morning (starting around 1100 h) and late in the afternoon or early evening (lasting until sunset) (Gibb *et al.*, 1998; Gregorini, 2012). Intake rate is greater during the afternoon than during the morning grazing sessions, primarily due to higher bite rate and larger bite mass (Gibb *et al.*, 1998; Taweel *et al.*, 2004). Thus, provision of supplements during major grazing periods (GP) may disrupt normal grazing activity thereby reducing daily grazing time, herbage DM intake and animal performance (Gekara *et al.*, 2005; Pulido *et al.*, 2009; Gregorini *et al.*, 2010).

Few studies have focussed on the importance of timing of supplementation on grazing dairy cow performance, and results have been variable. Sheahan *et al.* (2013) found that milk yield tended to increase when concentrate supplementation was offered to dairy cows in the morning after milking rather than in the afternoon, although the response was not associated with an increase in grazing time. However, Gekara *et al.* (2005) showed that intake rate and herbage DM intake were greater when lactating beef cows received concentrate in the morning rather than in the afternoon. Mitani *et al.* (2005) reported that milk protein yield and nitrogen retention were greater when dairy cows were offered a corn silage-based supplement before grazing rather than after grazing when the access time to pasture was restricted. With dairy cows provided access to pasture for only 5 h, Al-Marashdeh *et al.* (2016) reported that DM intake was greater when dairy cows received corn silage 9 h rather than 2 h before grazing, although no differences were found in milk and solid yields between treatments, changes in cow BW were decreased in the former treatment.

Our hypothesis was that by providing a corn silage supplement in two separate meals (before and after grazing) rather than offering it in only one meal (before or after the grazing session) would increase grazing time, herbage DM intake rate and total DM intake, and therefore milk production by dairy cows. The objective of this study was, therefore, to evaluate the effect of timing of corn silage supplementation on ingestive behaviour, DM intake, milk yield and composition in grazing dairy cows.

Materials and methods

Experimental design, animals and treatments

The experiment was carried out at the Experimental Research Station 'Dr M.A. Cassinoni' (EEMAC) of the School of Agronomy (Paysandú, Uruguay, 32°S, 58°W) in the late autumn and early winter period, with 1 week of adaptation (week 0) and 6 weeks of measurements (week 1 to 6). Animal procedures were approved by the Animal Experimentation Committee of the University of the Republic.

In total, 33 autumn-calving Holstein cows of 528 ± 31.5 kg (means ± SD) BW, body condition score (BCS) 2.35 ± 0.199, yielding 22.4 ± 3.49 kg milk/day at 48 ± 17.0 days in milk were selected. During the pre-experimental period, cows grazed a second-year mixed grass and legume pasture (one 8-h session/day), received 2.7 kg DM/day of a commercial concentrate at each milking and 3.8 kg DM/day of corn silage after PM milking. Animals were blocked by parity (2, 3 and 4 or more lactations), milk yield and days in milk and randomly assigned to one of the three treatments. The pasture grazed during the experiment was a second-year mixed pasture of tall fescue (*Festuca arundinacea*), white clover (*Trifolium repens*), bird's-foot trefoil (*Lotus corniculatus*), with a mean herbage mass measured to ground level of 1540 ± 176.4 kg DM/ha. Each treatment group had access to a daily strip of pasture between 0900 and 1500 h and received 2.7 ± 0.06 kg DM of a commercial concentrate at each milking (0430 and 1530 h). Whole-crop corn silage (3.8 ± 0.04 kg DM/day) was offered in a single meal at 0800 h (Treatment AM $n = 10$), equally distributed in two meals 0800 and 1700 h (Treatment AM-PM $n = 12$) or a single meal at 1700 h (Treatment PM $n = 11$) assigned in a randomized incomplete block design.

Daily strips of pasture for grazing were adjusted to provide a daily herbage allowance of 15 kg DM/cow (measured to ground level) based on the measurement of the pre-grazing herbage mass (kg DM/ha). The weights of concentrate and corn silage offered and refused were recorded on a daily basis to determine individual feed intake. Samples of the concentrate and corn silage, as offered, were collected every 14 days, dried at 60°C, and stored for subsequent analyses to determine chemical composition.

Cows were milked twice daily (0430 and 1530 h), and milk yields were recorded. Milk samples at each milking during 2 consecutive days per week were collected to determine milk fat, protein and lactose concentration with a MilkoScan (Model 133b; Foss Electric®, Hillerød, Denmark). Cow BCS in weeks 0, 2, 4 and 6 were estimated by visual observation using a five-point scale (Edmonson *et al.*, 1989) by the same observer.

Herbage mass and pasture depletion

To determine the appropriate strip areas, 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 the shortest, the tallest and three areas of intermediate sward height. At each location, sward plate height was measured to the nearest 0.5 cm using a rising plate meter (RPM; Ashgrove Co., Palmerston North, New Zealand) 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 in order to calculate herbage DM mass and derive a linear regression relating it to sward plate height. Every week, the herbage mass was estimated by measuring the sward plate height with the RPM at 50 points within the paddocks and applying the regression calculated at the start of the current or previous week. The temporal pattern of pasture height depletion during grazing was estimated twice weekly, during weeks 4, 5 and 6, by measuring sward height with the RPM at 1-h interval while the cows were at pasture (minimum of 30 points/strip per hour). During weeks 2, 4 and 6, aliquot samples of the herbage cut from each of the five locations within the three replicates in order to determine herbage mass were bulked and sub-samples taken to determine their chemical composition (Table 1).

Herbage dry matter intake

Individual herbage DM intake was determined in four cows per treatment (12 cows; four complete blocks) during 4 days in week 6 of the experiment, using *n*-alkanes (Dove and Mayes, 2006), with *n*-hentriacontane (*n*-C31) as an internal marker and *n*-dotriacontane (*n*-C32) dosed as an 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). Over the 8 days before, and during the 4 days of intake determination (total 12 days, weeks 5 and 6) cows were dosed with a cellulose bolus containing 342.5 ± 3.35 mg/day of *n*-alkane (*n*-C32) at each milking; thus, every cow received a daily dose of 685 mg/day. Herbage samples representing the forage selected by cows over the final 4 days were collected by hand plucking, from areas adjacent to the grazing plots followed by individual cows for 10 min every hour during the grazing sessions (Coates and Penning, 2000). They were combined, dried at 60°C and stored until they were analysed for concentration of *n*-alkanes (*n*-C31, *n*-C32 and *n*-C33). Faeces samples were collected from the rectum of each cow after every milking on the final 4 days of the measurement period and immediately stored frozen at −20°C until they were analysed. To correct for the contribution made by the supplements to the diet, samples of the concentrate and corn silage were collected during these final 4 days, before feeding, and used to determine their DM content and *n*-alkane profiles (Dove and Mayes, 2006).

Grazing and ruminating activity

Grazing and ruminating activity were determined visually by three trained observers in weeks 4, 5 and 6. On 3 consecutive days, the grazing or ruminating activities of 12 cows (the same four complete blocks used to estimate herbage DM intake) were recorded every 15 min (Chilibroste *et al.*, 2012). To examine temporal patterns of activity, data were collated within each successive 1-h observation period (OP1 to OP6). Bites rates were determined at the beginning and the end of weeks 4, 5 and 6 during three GP between 0900 and 1000 h (GP1), 1130 and 1230 h (GP2), and between 1400 and 1500 h (GP3). During each GP the observers counted the number of bites during 1 min (Chilibroste *et al.*, 2012) by block and when completed (every 15 min), the procedure was repeated in all blocks until the end of each GP.

Chemical composition

The hand-plucked samples of herbage collected during the period of intake determination and the faeces samples were combined for each cow before analyses. All pasture, supplement and faeces samples were dried at 60°C to constant weight and ground through a 1 mm sieve. Hand-plucked samples of herbage were collected and composed by treatments on a weekly bases to determine DM, ash, CP, NDF, and ADF content according to Association of Official Analytical Chemists (1990) and '*in vitro*' digestibility as described by Tilley and Terry (1963). The supplements were sampled and analysed as the herbage samples. The *n*-alkane concentration of pasture, supplement and faeces samples were determined following the procedures described by Dove and Mayes (2006).

Calculations and statistical analyses

Net energy for lactation (NE_L) was calculated as described by National Research Council (NRC) (2001). Milk energy output was calculated as NE_L (Mcal/day) = milk yield $\times$ [(0.0929 $\times$ fat %) + (0.0563$\times$ true protein %) + (0.0395 $\times$ lactose %)], using milk composition data derived weekly from analysis of the four consecutive samples (NRC, 2001). All statistical analyses were conducted using the SAS Systems program package (v. 9.2, SAS Institute Inc., Cary, NC, USA). Milk yield and composition and BCS were analysed in a mixed model with repeated measurements in time, using the MIXED procedure and a 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 treatment $\times$ week interaction (when $P < 0.20$) as fixed effects and blocks as random effects. Dry matter intake data were analysed with a model that included treatment and blocks as fixed and random effects, respectively.

The number of observations of grazing and ruminating made at 15 min intervals were analysed with GENMOD procedure with a binomial distribution and a model that included block, week, treatment, OP and their interaction. Individual records of grazing activity were used to define the duration of the first grazing session and were analysed using the MIXED procedure with a model that included week and treatment as a fixed effect and block and day as a random effect. The number of bites per min (bite rate), were analysed using the MIXED procedure with a model that included week, treatment, GP and the interaction treatment $\times$ GP as a fixed effect and block and day as random. Based on the measurements of herbage DM intake, grazing time and grazing bites at week 6, herbage intake rate was calculated, and differences between treatments analysed with a model that included treatment and block as fixed and random effects, respectively. Correlation and regression coefficients between ruminating time and corn silage intake before access time to pasture were analysed using the CORR and REG procedures. Within each week, depletion rate of RPM height while cows were at pasture was calculated using the following model: $y = a \times \exp^{(-kt)}$, where a is the initial pasture height (before grazing), k the fractional disappearance rate of the pasture and t the hour from the beginning 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. Least square means were separated using Tukey–Kramer tests ($\alpha = 0.05$), and means were considered to differ if $P \leqslant 0.05$ and tendencies were declared if $0.05 < P \leqslant 0.10$. Some methods were similar to those described by Mattiauda *et al.* (2013).

Results

Dietary component analyses

Results of the analyses of samples collected as representative of the herbage, concentrate and corn silage eaten are presented in Table 1.

Milk yield and composition, and cow body condition score

Results of ANOVA of milk and constituent yields, milk composition and BCS are shown in Table 2. There were no treatment effects on milk yield, or milk protein and lactose concentration and yields. However, overall mean fat percentage and yield were greater in AM-PM than in AM cows and were intermediate in PM cows. There were treatment $\times$ week interaction effects on fat percentage and yield, with within-week treatment effects occurring in weeks 3 and 6 (data not shown).

Mean 4% FCM yield was 1.6 kg/day higher ($P < 0.10$) in AM-PM than in AM cows and intermediate in the PM cows. There was a treatment $\times$ week interaction effect on FCM yield, with within-week treatment effects occurring only in weeks 3 and 6 of the experiment (Figure 1a). Despite changes in FCM yield and milk fat were no differences in milk energy output between treatments. Cow BCS was greater in AM-PM than AM cows and intermediate in PM cows, and showed a treatment

Table 1 *Mean ± SD chemical composition of herbage representative of that selected by the cows, and corn silage and concentrate offered to dairy cows*

	Herbage	Corn silage	Concentrate
DM (%)	18.3 ± 1.28	25.6 ± 0.10	90.1 ± 0.85
OM (%)	90.8 ± 0.79	93.0 ± 0.05	91.8 ± 0.21
CP (%)	22.5 ± 1.65	6.8 ± 0.16	17.3 ± 0.22
NDF (%)	30.7 ± 1.15	56.6 ± 0.40	24.8 ± 0.14
ADF (%)	20.3 ± 1.15	31.7 ± 0.17	9.9 ± 0.05
OM digestibility *in vitro* (%)	77.5 ± 1.50	74.5 ± 2.12	78.2 ± 1.20
Net energy lactation[1] (Mcal/kg DM)	1.67	1.45	1.78

DM = dry matter; OM = organic matter.
[1]Estimated from the equation of NRC (2001).

Table 2 *ANOVA of effect of treatment (T) and week (W) on milk yield, 4% fat-corrected milk (FCM) yield, estimated milk energy output, milk composition and body condition score (BCS) of strip-grazed dairy cows offered a daily ration of 3.8 kg dry matter of corn silage in a single meal at either 0800 h (AM) or 1700 h (PM), or in two equal meals at 0800 and 1700 h (AM-PM)*

	Treatments				*P* value		
	AM	AM-PM	PM	rSD	T	W	T × W
Animals (*n*)	10	12	11				
Yield (kg/day)							
Milk	25.2	25.7	24.7	0.62	0.31	<0.01	0.55
FCM	23.7^{y}	25.3^{x}	24.6^{xy}	0.84	0.10	<0.01	0.02
Fat	0.92^{b}	1.00^{a}	0.97^{ab}	0.035	0.05	<0.01	0.01
Protein	0.75	0.75	0.75	0.023	0.92	<0.01	0.36
Lactose	1.20	1.22	1.18	0.043	0.60	<0.01	0.73
Energy (Mcal/day)	17.4	18.4	17.9	0.60	0.22	<0.01	0.02
Composition (%)							
Fat	3.66^{b}	3.89^{a}	3.85^{ab}	0.072	0.04	<0.01	0.02
Protein	2.99	2.93	2.98	0.048	0.65	<0.01	0.08
Lactose	4.81	4.73	4.69	0.045	0.16	<0.01	0.85
BCS	2.37^{b}	2.52^{a}	2.42^{ab}	0.063	0.03	<0.01	0.02

^{a,b}Means within a row with different superscript differ significantly at $P < 0.05$.
^{x,y}Means within a row with the different superscript trend to differ at $P < 0.10$.

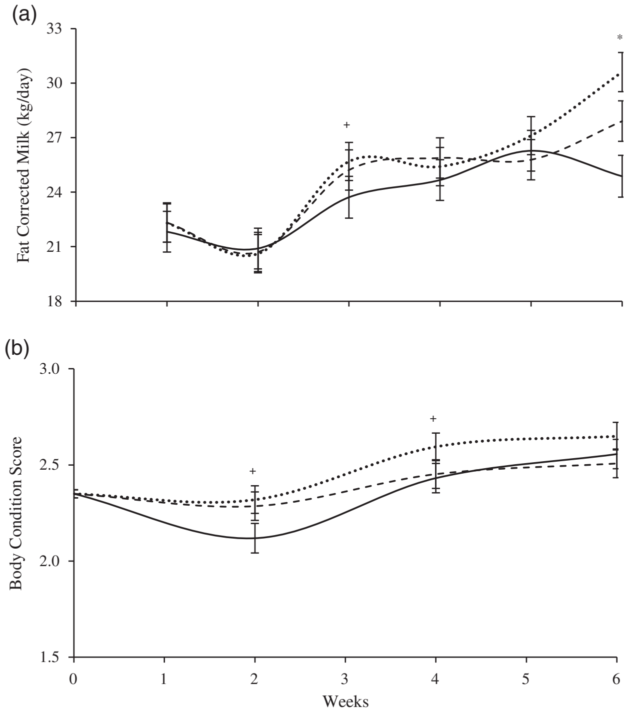

Figure 1 Milk yield 4% fat-corrected (a), and body condition score (b) of strip-grazed dairy cows offered corn silage at 0800 h (AM, ——), equally offered at 0800 and 1700 h (AM-PM, ••••••) or at 1700 h (PM, - - - -). Within a week, *indicates the difference ($P<0.05$), + indicates a tendency to be different ($P<0.10$).

× week interaction effect, with within-week treatment effects occurring in weeks 2 and 4 (Figure 1b).

Dry matter intake, grazing behaviour and pasture depletion
Mean daily DM intakes of herbage, corn silage and concentrate are shown in Table 3. Compared with the AM cows, treatment AM-PM cows achieved a greater daily herbage DM intake and, because the rations of silage and concentrate were entirely consumed by the cows, total intake of DM. Herbage and total daily DM intakes by PM cows were intermediate between those of AM-PM and AM cows.

The overall proportion of observations during which cows were grazing tended to be different between treatments ($P=0.06$; Table 4). However, there were differences between the 1-h OP, but there was no interaction between treatment × OP. The overall proportion of observations, during which cows were observed ruminating differed between treatments being AM ⩾ AM-PM > PM cows and there was an effect of OP (Table 4).

The proportion of observations during which cows were grazing was the highest in OP1, and the lowest in OP2, being intermediate in OP3, OP4 and OP5, and also intermediate but lower than OP3 to OP5 in OP6. The proportion of observations during which cows were grazing was greater in AM-PM than PM cows in OP1 and OP5, while differences were inverse in OP6 (Figure 2).

There was no treatment effect on the duration of the first grazing meal (73, 85 and 76 ± 8.1 min for treatments AM, AM-PM and PM, respectively). The number of observations of ruminating activity whilst at pasture was linearly correlated ($r=0.72$, $P<0.01$) with corn silage intake immediately before accessing the pasture. Assuming a ruminating bout duration of 15 min corresponding to each ruminating observation, linear regression analysis showed an increase of 7 min for each kg DM intake of corn silage before turnout; $y=23.9+7.1\ x$ where y is ruminating time (min) and x the corn silage DM intake before grazing session.

Table 3 *Daily dry matter (DM) intake by strip-grazed dairy cows offered a daily ration of 3.8 kg DM of corn silage in a single meal at either 0800 h (AM) or 1700 h (PM), or in two equal meals at 0800 and 1700 h (AM-PM)*

	Treatments				
	AM	AM-PM	PM	rSD	*P* value
Animals (*n*)	10	12	11		
DM intake (kg)					
Herbage[1]	8.5^{b}	11.0^{a}	10.3^{ab}	0.68	0.03
Corn silage	3.9	3.8	3.9	0.04	–
Concentrate	5.3	5.3	5.3	0.06	–
Total	17.7^{b}	20.1^{a}	19.5^{ab}	0.68	0.032

[1]Herbage intake was estimated in four animals per treatment.
[a,b]Means within a row with different superscript differ significantly at $P<0.05$.

Mean bite rate was not affected by treatments (Table 4) but differed between the first second and third period of measurement: 51, 44 and 43 ± 1.3 bites/min in GP1, GP2 and GP3, respectively. Intake rate was greater in AM-PM than AM cows and intermediate for PM cows: 31.0, 41.5 and 39.6 ± 5.44 g DM/min in AM, AM-PM and PM; respectively. The hourly measurements of RPM height showed no treatment differences in the rate of height reduction. The fractional rate of height reduction in the three treatments could be represented by the joint expression $y=31.52\exp^{(-0.01\,t)}$ ($R^2=0.77$), where y is the pasture RPM height, and t the time (h) since the cows entered the pasture.

Discussion

In the conditions of this experiment, where the time spent at pasture was restricted, dividing the corn silage supplement between two meals increased dairy cows' performance (milk fat percentage and yield, FCM yield and BCS) as stated in our hypothesis. The difference in FCM yield between the AM-PM and AM cows tended to be different, with that of the PM cows being intermediate. Mitani *et al.* (2005) similarly reported an increase in milk fat percentage when a corn silage-based supplement (in a ratio 70 : 30 corn silage : concentrate) was offered to dairy cows after grazing compared with before grazing, even although the supplement was offered as a mixed ration and cows had two sessions at pasture over the day. In contrast, Al-Marashdeh *et al.* (2016) found no differences in milk yield or composition when corn silage, similar to that offered in the present experiment, was offered 2 or 9 h before grazing to cows at an advanced stage of lactation (>28 weeks). Trevaskis *et al.* (2004)

Table 4 *ANOVA of effect of treatment (T) on the proportion of observations in experimental weeks 4, 5 and 6, in which strip-grazed dairy cows were grazing or ruminating during six consecutive 1-h observation periods (OP), and on bite rates counted during three non-consecutive 1-h grazing periods (GP) (0900 to 1000 h, 1130 to 1230 h and 1400 to 1500 h)*

	Treatments				*P* value		
	AM	AM-PM	PM	rSD	T	OP	T × OP
Animals (*n*)	4	4	4				
Proportion of 1-h period							
Grazing	0.74[y]	0.78[x]	0.71[y]	0.525	0.06	0.04	0.35
Ruminating	0.09[a]	0.06[a]	0.03[b]	0.014	<0.01	<0.01	–
					T	GP	T × GP
Bite rate (bites/min)	47	45	46	1.3	0.19	<0.01	0.63

The three treatments consisted of cows being offered a daily ration of 3.8 kg DM of corn silage in a single meal at either 0800 h (AM) or 1700 h (PM), or in two equal meals at 0800 and 1700 h (AM-PM).
[a,b]Means within a row with different superscript differ significantly at $P < 0.05$.
[x,y]Means within a row with the different superscript trend to differ at $P < 0.10$.

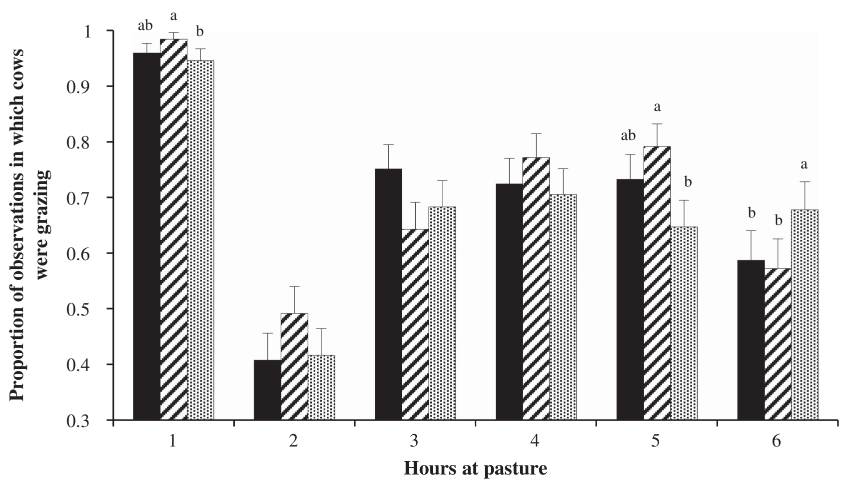

Figure 2 The proportion of visual observations in each hour at pasture during which cows were grazing, when offered corn silage either in a single meal before (AM, ■), in two equal meals before and after (AM-PM, ▨) or in a single meal (PM, ▦) after 6 h at pasture. [a,b]Columns within an hour with different letter differed at $P < 0.05$.

and Sheahan *et al*. (2013) reported no differences in milk yield or composition due to the timing of concentrate supplementation when cows were at pasture for 24 h. Such contrasting results between experiments probably result from differences in the type of supplement (roughage *v*. concentrate), fasting time before grazing and the time allowed at pasture (5 or 6 h *v*. 24 h) affecting herbage DM intake and the interaction between the dietary components (Chilibroste *et al*., 2015). Cow BCS, as an approximate indicator of body reserves and energy balance (Meikle *et al*., 2013), was greater in AM-PM than AM cows and intermediate in PM cows in contrast with the results reported by Al-Marashdeh *et al*. (2016) where they found no changes in BCS between supplements treatments. The greater FCM yield and BCS in AM-PM cows than in the other treatments could be explained, at least partially, by a greater total DM intake due to greater herbage DM intake.

Herbage DM intake measured using the *n*-alkane technique showed that cows on treatment AM consumed less herbage than those on AM-PM, whereas herbage DM intake by cows receiving their full corn silage ration after grazing was intermediate between the other two treatments. In contrast to our results, Mitani *et al*. (2005) reported no differences in herbage DM intake due to the timing of supplementation when dairy cows had 5 h total access time split between two sessions at pasture. Similarly, cows allowed 24 h access to pasture, either with (Sheahan *et al*., 2013) or without (Trevaskis *et al*., 2004) fresh allocation of pasture, showed no effect of supplement timing on herbage DM intake. Probably, in these latter experiments, the greater number of grazing sessions or longer time on pasture, in comparison to the present study, allowed cows to compensate for the effect of timing of supplementation on herbage DM intake as was reported by Gibb *et al*. (2000). Al-Marashdeh *et al*. (2016) reported that similar to our study, herbage DM intake was lower when corn silage was offered 2 *v*. 9 h before the grazing session. The reduced DM intake in AM cows could be explained by the greater rumen fill hastening satiation (Gregorini *et al*., 2009; Chilibroste *et al*., 2015) and the observed predisposition towards enhanced ruminating activity. On the other hand, although PM cows should present a lower rumen fill and conversely higher herbage DM intake, a lower ruminating during ingestion due to the higher intake rate exhibited by this treatment (Chilibroste *et al.*, 2007) might have determined a less stable rumen environment with negative effects on rumen fermentation and microbial biomass growth (Chilibroste *et al.*, 2008).

Ruminants, particularly dairy cows, show a grazing pattern with a daily frequency of three to five grazing events with major meals occurring during the early and late morning and late in the afternoon and evening (Gibb *et al.*, 1999). This pattern is flexible and is influenced by the environment as well as responding to behavioural adaptations to animal husbandry and grazing management (Gibb, 2006; Gregorini, 2012). In this study, the restricted access time to pasture (6 h between morning and afternoon milking) limited the ability of cows to express a more natural temporal pattern of daily grazing activity, because the major grazing event that would normally occur in the late afternoon and early evening was prevented. The timing of corn silage supplementation could further impact on grazing behaviour and herbage DM intake as it might establish a different internal state (hunger or satiation stimuli) at the first and important grazing session, which changes cow reaction to the perception of the same feed resource (Gregorini *et al.*, 2009).

Providing the entire corn silage ration just before access to pasture (AM cows) may have directly affected the grazing process, due to satiety stimuli coming from the interaction between ingestive and digestive behaviour affecting short-time DM intake (Gregorini, 2012). In contrast, when the same amount of supplement was offered and consumed in two meals (AM-PM), fermentation pattern and rumen environment could have been being more stable (Chilibroste *et al.*, 2008), which might have stimulated more intensive grazing activity and hence greater herbage DM intake (Gregorini, 2012).

Contrary to the differences in herbage DM intake measured using the *n*-alkane technique, treatments did not affect the duration of the first grazing session. In retrospect, the failure to detect significant differences in overall grazing activity (despite the tendency for higher grazing activity in AM-PM that in the other treatments) was probably due to the excessive interval (15 min) between observations, during which treatment differences in inter- and intra-meal intervals may not have been detected. Nevertheless, during the 1st h at pasture there was a small but significant treatment effect on the proportion of observations when cows were grazing, being higher in treatment AM-PM than PM and intermediate for AM cows. Due to the limited total time of 6 h that cows were allowed at pasture, the large proportion of cows devoted to grazing during the 1st h at pasture (OP1) fulfil a major contribution to total daily herbage DM intake (Chilibroste *et al.*, 2015). During the 2nd h at pasture the incidence of grazing activity was very much reduced, being replaced to a large extent by increased ruminating activity (data not shown). During OP4 and OP5, cows were recorded grazing in more than 0.6 of observations, with a higher proportion occurring in AM-PM cows than in PM cows (Figure 2). In the last hourly OP6 grazing activity declined slightly in the AM and AM-PM cows, whereas PM cows grazed more than the previous one. As in the present experiment, but where cows were allowed access to pasture for 12 or 24 h, Trevaskis *et al.* (2004) and Gekara *et al.* (2005) reported no effect of timing of concentrate meal (morning *v.* afternoon) on total grazing time by lactating dairy and beef cows, respectively. Sheahan *et al.* (2013) similarly found no difference in total grazing time when dairy cows were offered 3 kg of concentrate either in the morning or afternoon, but did report that the morning grazing bout tended to be longer when cows received their supplement in the afternoon. Gibb *et al.* (2000) did not report differences neither in grazing nor in ruminating time when cows had access to 8 kg of concentrate twice a day in or out of parlour, but the number of grazing meals were greater for cows receiving concentrate out of parlour which supports the opportunities to modify ingestive behaviour through the use of timing of supplementation.

Ingestive behaviour determines herbage DM intake as a function of grazing time, bite mass and bite rate (Hodgson, 1985; Chilibroste *et al.*, 2007). In a review of the effect of supplements on cows at pasture, Bargo *et al.* (2003) reported that supplementation often reduces grazing time, but does not affect bite rate or bite mass. Although short-term herbage DM intake rate can be significantly affected by the time of day (Gibb *et al.*, 1998) and cow physiological state (Gibb *et al.*, 1999), it is primarily influenced by sward state, which constrains bite mass and in turn bite rate (Gibb, 2006). In the present study, measurements of the fractional reduction in RPM height while the cows were at pasture showed no difference between treatments, suggesting little or no effect on pasture structure modification. Nevertheless, cows have been shown to increase bite mass in response to decreased rumen fill (Chilibroste *et al.*, 2000; Gregorini *et al.*, 2007). Thus, the significantly greater herbage DM intake by the AM-PM compared with the AM cows may have been achieved by the combined effect of small increases in bite mass and total grazing activity in response to their reduced rumen fill at the beginning of the grazing session. The amount of supplement consumed before being released to pasture had an undeniable impact on ruminating activity during much of the following 6 h; estimated ruminating time to increase by 7 min for each kilogram DM of corn silage consumed at the morning feed. This increased ruminating activity by AM cows was consistent with previous reports that, compared with unsupplemented cows, those receiving supplements increased the time they spent ruminating (Sheahan *et al.*, 2011), and performed longer and more frequent ruminating bouts (Pérez-Prieto *et al.*, 2011). Sheahan *et al.* (2013) observed in dairy cows with 24 h access to pasture, that total ruminating time was greater when concentrate was supplemented in the afternoon rather than in the morning, due to increased ruminating during darkness. In our work, ruminating time was only measured during access time to pasture, which is probably not representative of rumination during the whole day. Whilst at pasture the time budgets for grazing and ruminating activity by the cows was constrained by the limited time available, so that the requirement for rumination and comminution of corn silage particles, albeit limited, impacted on grazing activity. Following afternoon milking, without the opportunity to graze, ruminating activity could have been far less constrained.

Conclusions

In the conditions of this experiment, with restricted access to pasture, cows receiving corn silage supplementation in two meals or a single meal after grazing increased DM intake, FCM yield and BCS compared with cows receiving it before grazing. A larger input (determined) and better synchronization of nutrients at rumen level (speculated) can be postulated as the main factors involved in the animal performance response. Both factors were mediated by changes in animal ingestive behaviour.

Acknowledgements

Special thanks to Dra. A. Meikle for her support and revision of this manuscript. The authors acknowledge Ing.Agr. F. Elizondo for his assistance throughout the field work. The first author would like to thank Elena Chilibroste for the edition of the manuscript and recognize to A.L. Astessiano and A. Casal for their assistance with the edition.

Declaration of interest

Authors do not have any actual or potential conflict of interest; financial, personal or other relationships with other people or organizations.

Ethics statement

This experiment received ethical approval from the Animal Experimentation and Ethical Committee of the University of the Republic.

Software and data repository resources

Data are not deposited in an official repository.

References

Al-Marashdeh O, Gregorini P and Edwards GR 2016. Effect of time of maize silage supplementation on herbage intake, milk production, and nitrogen excretion of grazing dairy cows. Journal of Dairy Science 99, 7123–7132.

Association of Official Analytical Chemists 1990. Official methods of analysis, 15th edition. AOAC, Arlington, VA, USA.

Armstrong DP, Tarrant KA, Ho CKM, Malcolm LR and Wales WJ 2010. Evaluating development options for a rain-fed dairy farm in Gippsland. Animal Production Science 50, 363–370.

Bargo F, Muller LD, Kolver ES and Delahoy JE 2003. Invited review: production and digestion of supplemented dairy cows on pasture. Journal of Dairy Science 86, 1–42.

Coates DB and Penning P 2000. Measuring animal performance. In Field and laboratory methods for grassland and animal production research (ed. L `tMannetje and RM Jones), pp. 353–402. CABI Publishing, Wallingford, UK.

Chilibroste P, Dijkstra J, Robinson PH and Tamminga S 2008. A simulation model 'CTR Dairy' to predict the supply of nutrients in dairy cows managed under discontinuous feeding patterns. Animal Feed Science and Technology 143, 148–173.

Chilibroste P, Gibb MJ, Soca P and Mattiauda DA 2015. Behavioural adaptation of grazing dairy cows to changes in feeding management: Do they follow a predictable pattern? Animal Production Science 55, 328–338.

Chilibroste P, Gibb MJ and Tamminga S 2005. Pasture characteristics and animal performance. In Quantitative aspects of ruminant digestion and metabolism (ed. J Dijkstra, JM Forbes and J France), pp. 681–706. CABI Publishing, Wallington, UK.

Chilibroste P, Mattiauda DA, Bentancur O, Soca P and Meikle A 2012. Effect of herbage allowance on grazing behavior and productive performance of early lactation primiparous Holstein cows. Animal Feed Science and Technology 173, 201–209.

Chilibroste P, Soca P, Mattiauda DA, Bentancur O and Robinson PH 2007. Short term fasting as a tool to design effective grazing strategies for lactating dairy cattle: a review. Australian Journal of Experimental Agriculture 47, 1075–1084.

Chilibroste P, Tamminga S, Boer H, Gibb MJ and den Dikken G 2000. Duration of regrowth of ryegrass (Lolium perenne) effects on grazing behavior, intake, rumen fill, and fermentation of lactating dairy cows. Journal of Dairy Science 83, 984–995.

Dove H and Mayes RW 2006. Protocol for the analysis of n-alkanes and other plant-wax compounds and for their use as markers for quantifying the nutrient supply of large mammalian herbivores. Nature Protocols 1, 1680–1697.

Edmonson A, Lean I, Weaver L, Farver T and Webster G 1989. A body condition scoring chart for Holstein dairy cows. Journal of Dairy Science 72, 68–78.

Gekara OJ, Prigge EC, Bryan WB, Nestor EL and Seidel G 2005. Influence of sward height, daily timing of concentrate supplementation, and restricted time for grazing on forage utilization by lactating beef cows. Journal of Animal Science 83, 1435–1444.

Gibb MJ 2006. Grassland management with emphasis on grazing behaviour. In Fresh herbage for dairy cattle (ed. A Elgersma, J Dijkstra and S Tamminga), pp. 141–157. Springer, Dordrecht, The Netherlands.

Gibb MJ, Huckle CA and Nuthall R 1998. Effect of time of day on grazing behaviour by lactating dairy cows. Grass and Forage Science 53, 41–46.

Gibb MJ, Huckle CA and Nuthall R 2000. Effect of temporal pattern of supplementation on grazing behaviour and herbage intake by dairy cows. In Grazing management: the principles and practice of grazing, for profit and environmental gain, within temperate grassland systems (ed. AJ Rook and PD Penning), pp. 91–96. British Grassland Society, Reading, UK.

Gibb MJ, Huckle CA, Nuthall R and Rook AJ 1999. The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Applied Animal Behaviour Science 63, 269–287.

Gregorini P 2012. Diurnal grazing pattern: its physiological basis and strategic management. Animal Production Science 52, 416–430.

Gregorini P, Clark CEF, Jago JG, Glassey CB, McLeod KLM and Romera J 2009. Restricting time at pasture: effects on dairy cow herbage intake, foraging behavior, hunger-related hormones, and metabolite concentration during the first grazing session. Journal of Dairy Science 92, 4572–4580.

Gregorini P, Gunter SA, Masino CA and Beck PA 2007. Effects of ruminal fill on short-term herbage intake rate and grazing dynamics of beef heifers. Grass and Forage Science 62, 346–354.

Gregorini P, Soder KJ and Waghorn G 2010. Effects of timing of corn silage supplementation on digestion, fermentation pattern, and nutrient flow during continuous culture fermentation of a short and intensive orchardgrass meal. Journal of Dairy Science 93, 3722–3729.

Haydock K and Shaw N 1975. The comparative yield method for estimating dry matter yield of pasture. Australian Journal of Experimental Agriculture 15, 663–670.

Hills JL, Wales WJ, Dunshea FR, Garcia SC and Roche JR 2015. Invited review: an evaluation of the likely effects of individualized feeding of concentrate supplements to pasture-based dairy cows. Journal of Dairy Science 98, 1363–1401.

Hodgson J 1985. The control of herbage intake in the grazing ruminant. Proceedings of Nutrition Society 44, 339–346.

Mattiauda DA, Tamminga S, Gibb MJ, Soca P, Bentancur O and Chilibroste P 2013. Restricting access time at pasture and time of grazing allocation for Holstein dairy cows: ingestive behaviour, dry matter intake and milk production. Livestock Science 152, 53–62.

Meikle A, Adrien ML, Mattiauda DA and Chilibroste P 2013. Effect of sward condition on metabolic endocrinology during the early postpartum period in primiparous grazing dairy cows and its association with productive and reproductive performance. Animal Feed Science and Technology 186, 139–147.

Mitani T, Takahashi M, Ueda K, Nakatsuji H, Kondo S and Okubo M 2005. Effects of supplementary corn silage on the feed intake and milk production of time-restricted grazing dairy cows. Animal Science Journal 76, 331–337.

National Research Council (NRC) 2001. Nutrient requirements of dairy cattle, 7th revised edition. National Academic Science, Washington, DC, USA.

Pérez-Prieto LA, Peyraud JL and Delagarde R 2011. Substitution rate and milk yield response to corn silage supplementation of late-lactation dairy cows grazing low-mass pastures at 2 daily allowances in autumn. Journal of Dairy Science 94, 3592–3604.

Peyraud JL and Delagarde R 2013. Managing variations in dairy cow nutrient supply under grazing. Animal 7, 57–67.

Pulido RG, Muñoz R, Lemarie P, Wittwer F, Orellana P and Waghorn GC 2009. Impact of increasing grain feeding frequency on production of dairy cows grazing pasture. Livestock Science 125, 109–114.

Sheahan AJ, Gibbs SJ and Roche JR 2013. Timing of supplementation alters grazing behavior and milk production response in dairy cows. Journal of Dairy Science 96, 477–483.

Sheahan AJ, Kolver ES and Roche JR 2011. Genetic strain and diet effects on grazing behavior, pasture intake, and milk production. Journal of Dairy Science 94, 3583–3591.

Smit H, Taweel HZ, Tas BM, Tamminga S and Elgersma A 2005. Comparison of techniques for estimating herbage intake of grazing dairy cows. Journal of Dairy Science 88, 1827–1836.

Taweel HZ, Tas BM, Dijkstra J and Tamminga S 2004. Intake regulation and grazing behavior of dairy cows under continuous stocking. Journal of Dairy Science 87, 3417–3427.

Tilley JMA and Terry RA 1963. A two-stage technique for the in vitro digestion of forage crops. Journal of British Grassland Society 18, 104–111.

Trevaskis LM, Fulkerson WJ and Nandra KS 2004. Effect of time of feeding carbohydrate supplements and pasture on production of dairy cows. Livestestock Production Science 85, 275–285.