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Use of mixed rations with different access time to pastureland on productive responses of early lactation Holstein cows

M. Fajardo [a,*], D.A. Mattiauda [a], G. Motta [a], T.C. Genro [b], A. Meikle [c], M. Carriquiry [a], P. Chilibroste [a]

[a] Facultad de Agronomía, Departamento de Producción Animal y Pasturas, Universidad de la República, Montevideo, Uruguay
[b] Embrapa Pecuária Sul, Bagé, RS, Brasil
[c] Facultad de Veterinaria, Departamento de Biología Molecular y Celular, Universidad de la República, Montevideo, Uruguay

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ABSTRACT

The intensification of milk production has required the development of new feeding strategies such as the use of mixed rations either with or without access to grazing. The aim of this study was, to determine milk production and composition, body condition score (BCS), live weight (LW) and grazing behaviour of Holstein dairy cows subject to diets combining mixed rations and herbage during their first 10-wk of lactation (period 1). In addition, both the behavioural adaptation and productive response of cows fed 100% mixed ration when returning to grazing for a 20 days period were examined (period 2). An incomplete randomized block design was used, with 41 early lactation multiparous cows, assigned to treatments: G0 (confined cows fed 100% mixed ration), G1 (6 h access to paddocks + 50% mixed ration) and G2 (9 h access to paddock + 50% mixed ration). In a second period (11 to 13- wk of lactation), G0 (Post-TMR) cows were managed as G1, with G1 as the control treatment. During the first period, G0 cows produced more milk (37.2 *vs* 33.7 and 33.9 ± 0.92 L/d; $P<0.0001$) and more protein (1.25 *vs* 1.12 and 1.13 ± 0.038 kg/d; $P=0.006$) than G1 and G2. Nevertheless, no significant differences in fat and lactose production, and output of milk energy were found between G0 and G2 cows. With more access time to the paddock G2 cows could express a higher herbage dry matter intake (DMI) than G1 cows through a higher total grazing time. However, no differences were found on milk production and composition between G1 and G2 cows, probably due to a higher maintenance energy requirement for G2 cows. In period 2 no significant differences were detected in milk production, milk fat and protein production, BCS or LW between Post-TMR and G1 cows. Also, grazing behaviour was not different between G1 and Post-TMR cows, suggesting a prompt adaptation of G0 cows to grazing. It can be concluded that 100% mixed ration systems had higher milk production than grazing plus TMR systems in early lactation. An increase in access time to pastureland allowed higher herbage intake, although with no significant differences in milk energy output. The use of fresh pasture to replace mixed rations after the first 65 DIM allowed maintaining high milk and solids production.

1. Introduction

The intensification of grazing dairy systems has led to an increase on the use of supplements and more recently on the use of total mixed rations (TMR) (Wales et al., 2013), particularly when imbalances between herbage production and herd requirements occur during the course of a year (Chilibroste et al., 2007; Wales et al., 2013). Increased levels of TMR fed to grazing dairy cows have resulted in higher levels of milk production, where cows fed 100% TMR produced ~49% more milk than cows grazing without supplementation (Kolver and Muller, 1998) and ~17% more milk than grazing cows with access to TMR (Bargo et al., 2002; Vibart et al., 2008). However, such apparently large responses to supplementary feeding may have been due to limitations on dry matter intake (DMI) at pasture and to increasing maintenance energy requirements as result of more intensive walking required by grazing (Bargo et al., 2002). Nonetheless, those responses will also depend on TMR composition as well as the quality and quantity of herbage offered (Wales et al., 2013).

Previous research we have carried out has also shown increased milk yields by cows fed TMR compared with grazing. Sprunck et al. (2012) reported that multiparous cows offered a TMR *ad libitum* increased milk by 30% compared with grazing cows provided with

* Correspondence to: EEMAC, Ruta 3, km 363, Paysandú CP 60000, Uruguay.
E-mail address: maitefs@gmail.com (M. Fajardo).

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8.5 kg DM of the mixed ration per day and ample herbage allowance. Similarly, Meikle et al. (2013) reported that in primiparous cows a TMR that was offered *ad libitum* increased milk yield by approximately 5%, 10% and 25% compared with grazing cows provided with 15.2 kg (on a fresh weight basis) of TMR plus a daily herbage DM allowance of 30, 15 and 7.5 kg, respectively.

Production responses to feeding strategies based on combinations of herbage and TMR during early lactation will be influenced by grazing behaviour (Chilibroste et al., 2012), DMI and rumen fermentation patterns (Greenwood et al., 2014). Chilibroste et al. (2012) reported that during the first three weeks of lactation, primiparous dairy cows spent a relatively low proportion of time grazing whilst at pasture (< 35%), irrespective of the herbage allowance provided. However, Chilibroste et al. (2007) and Kristensen et al. (2007) had previously reported that dairy cows in mid lactation increased their total grazing time as result of having more access time to pasture. It therefore seems reasonable to suppose that if access time to pasture were increased in early lactation, then grazing time and as a consequence dry matter intake could also be increased.

Few studies have focused on the adaptation of dairy cows given a TMR when returning to a feeding regime involving grazing. Cows have various means by which they can adapt to new diets and/or changes in feeding management (Peyraud et al., 1996; Chilibroste et al., 2012). In grazing dairy cows, these include changes in the mechanics and temporal patterns of grazing to allow modifications of intake rate to compensate for variations in food availability and thereby maintaining DMI and animal performance (Chilibroste et al. ,2007; Kristensen et al., 2007; Mattiauda et al., 2013). Therefore, a better understanding of the effects of different feeding strategies on animal performance will foster improvements in actual feeding practices.

A study was conducted to compare the performance of multiparous dairy cows during the first 10-wk of lactation when fed either a TMR *ad libitum* without grazing, or a mixed ration at a level equivalent to 50% of that achieved *ad libitum* and while being allowed access to pastureland for either 6 or 9 h each. After the completion of the 10-wk measurement period, cows previously offered TMR *ad libitum* were provided a reduced (50%) daily allowance of mixed ration and were allowed access to pastureland for 6 h day^{-1} for a further measurement period of 20 days.

The hypothesis tested were (1) that cows fed 100% TMR (without access to pasture) during the first 10-wk of lactation would achieve a greater total DMI, milk production, body condition score (BCS) and live weight (LW) than cows with access to pastureland (6 or 9 h); (2) increasing the daily access time to pastureland (from 6 to 9 h) would lead to an increase in pasture DMI and milk production and changes in milk composition; (3) the transition from a 100% TMR to 50% TMR with 6 h access to pastureland would allow the cows to graze and achieve a higher total DMI, compared with cows already adapted to this feeding regime.

2. Materials and methods

2.1. Cows and pre-calving management

Forty one multiparous Holstein cows from the dairy herd of the Experimental Station Dr. M.A. Cassinoni (EEMAC) of the Agronomy Faculty were used in a 13-wk study from 19 March to 17 June 2011. Cows were blocked by parity, expected calving date (ECD), BCS and LW. The cows had a mean parity of 4.5 ± 1.81, and mean BCS and LW of 3.2 ± 0.23 and 734 ± 69.6, respectively, measured 3–5 days prior to calving.

During weeks 8–4 prior to their ECD, the herbage offered to cows was regulated to maintain a BCS at calving of between 3.0 and 3.5 (on a 5-point scale; Edmonson et al. 1989). During the last 4 weeks of gestation, cows were offered a mixed diet, based on 20 kg (on a fresh weight basis) of whole crop maize silage, 4 kg on a pre-calving commercial ration and hay *ad libitum*.

The experimental protocol was evaluated and approved by the Honorary Committee for Animal Experimentation in Uruguay (CHEA –UdelaR, Montevideo, Uruguay).

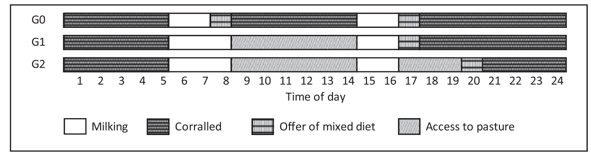

Fig. 1. Temporal pattern of management for cows offered either a mixed ration *ad libitum* (G0), or a restricted amount (50%) of the same mixed diet offered to G0 plus an allowed access to pasture for either 6 (G1) or 9 (G2) hours daily.

2.2. Experimental design

The experiment was an incomplete randomized block design comprising two periods. Cows calved between 27 February and 20 April were allocated the day after calving to one of three treatments that took place until 27 May (period 1). From 28 May until 17 June, a common feeding regime was imposed on two groups of cows which had previously received different feeding management (period 2).

During the first period cows were randomly assigned to one of the following treatments (Fig 1):

G0: Cows were offered a TMR *ad libitum* immediately after each milking on a feed pad without access to pastureland.

G1: Cows were allowed access to pastureland for 6 h each day and received an allocation of the mixed diet, equivalent to half the daily offered on G0, after the pm milking.

G2: Cows were allowed access to pastureland for 9 h each day and received an allocation of the mixed diet, equivalent to half the daily offered on G0, after the pm milking.

During the second experimental period cows previously allocated to treatment G0 were allowed access to a separate paddock for a period of 6 hours daily and received the mixed diet at half the rate previously offered. Thus, the new treatment (referred to as Post-TMR) was the same as the G1 treatment regime.

2.3. Feeding and grazing management

From the beginning of the experiment until 31 May, the TMR was composed, on a DM basis, of maize silage (0.45), dry ground maize (0.19), wheat grain (0.12), sunflower expeller (0.11), soybean expeller (0.09), urea (0.003), vitamins and minerals (0.009). From 1 June maize silage was replaced by sorghum silage as a fibre source in the TMR because the supply of maize silage had run out.

The mixed ration for treatment G0 was formulated according to NRC (2001) with a milk production target of 40 kg/d. The diet was offered *ad libitum*, adjusted at weekly intervals, at a rate 15% in excess of mean daily DMI measured the previous week. Treatments G1 and G2 had the same total daily offer of DM as G0, but differing on diet source: 50% of DM was offered as TMR and 50% as herbage. Herbage allowance was adjusted weekly altering the paddock area, based upon measurements of the herbage mass (kg DM/ha).The mixed diet was offered in open stalls with group feed trough with continuous access to water. During the period that herbage intake was measured, four cows from each treatment were selected, based upon parity, BCS, LW and days in milk (DIM) and fed individually to measure DMI of the mixed ration. The daily routine for cows on each treatment is shown in Fig. 1.

Cows on G1 and G2 treatments grazed a second-year pasture of *Festuca arundinacea*, *Trifolium repens* and *Lotus corniculatus*, located 1.7 km from the milking parlour with access to water in the pastureland. A new grazing area was assigned weekly in a 7-day rotational system, providing a mean herbage allowance of 15 kg DM/cow/d (4 cm above ground level) during period 1 and 18 kg DM/cow/d in period 2.

Herbage mass (kg DM/ha) was estimated weekly using a comparative yield method adapted from Haydock and Shaw (1975) with a 5-point calibration scale and 3 replicates for each category. The sward height at each of the fifteen calibration sites was measured using a rising plate metre, before cutting the herbage to ground level. Sward height was then measured at 300 randomly chosen sites with the rising plate metre and, using the regression derived from the fifteen calibration quadrats, the mean herbage mass was calculated for the pasture. This value was used to calculate the area required to provide the designated herbage allowance.

2.4. Sampling procedures and laboratory analysis

Individual milk yield was measured at each milking with a Waikato© milk metre (2008 Waikato Milking Systems USA Limited). Fat, protein and lactose concentrations in milk were determined weekly in two consecutive milkings by mid-infrared spectrophotometry (NIRS, Milko-Scan, Fross Electric, HillerØd, Denmark). Output of milk energy was estimated according to the following equation (NRC, 2001):

$$\text{Milk Energy (Mcal/d)} = ((0.0929^{*}\text{kg fat}) + (0.0547^{*}\text{kg protein}) + (0.0395^{*}\text{kg lactose}))^{*}\ 100$$

and was converted to MJ/d with the equivalence 1 Mcal = 4.1868 MJ.

Live weight and BCS were determined weekly after morning milking without fasting and BCS was always determined by the same person.

2.5. Chemical composition and dry matter intake

Ration components were analysed by near-infrared spectroscopy (methods 167.03, 42.05 and 984.13; AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured sequentially (Van Soest et al., 1991; without sodium sulphite in the neutral detergent solution) using an ANKOM200 Fibre Analyzer (ANKOM Technology Corp., Fairport, NY, USA). NDF was assayed without a heat stable amylase. Both fibre contents were expressed inclusive of residual ash.

To estimate herbage DMI the n-alkanes technique (Dove and Mayes, 2006) was used in period 1 (24 ± 10 DIM; weeks 4 and 5) and period 2 (83 ± 11 DIM; weeks 12 and 13). Alkanes were administered to cows twice every day after each milking for 12 consecutive days in cellulose pellets with a bolus gun to 10 cows on each treatment. Faecal samples were collected after each milking from days 6 to 12 of alkane administration period and immediately frozen at −20 °C, later thawed and dried in a forced air oven at 60 °C, and milled to pass through a 2 mm screen. Samples of the herbage representative of that eaten by the cows were hand clipped in each treatment at the approximate height to which cows grazed in each treatment on the previous paddock. The samples were dried at 60 °C in a forced air oven and milled to pass through a 2 mm screen before analysis of DM, alkane contents and nutritive characteristics (Table 1). Herbage and mixed diet NEl (MJ/kgDM) were calculated using to the following formula (Mieres, 2004):

$$\text{Herbage NEl (MJ/kg DM)} = (2,301 - (0,0289 \times \%\text{ADF}))^{*}4.1868$$

$$\text{Mixed diet NEl (MJ/kg DM)} = (1,909 - (0,015 \times \%\text{ADF}))^{*}4.1868$$

Composite samples of the faeces collected from each cow over the 7 days were analysed at the Animal Nutrition Laboratory (Embrapa Southern Region Animal Husbandry, Bagé, RS, Brazil). Alkane extraction and determination of the faeces and herbage samples were conducted according to the method described by Dove and Mayes (2006). The derivative equation proposed by Dove and Mayes (1991), which takes into account the intake of supplements was used to estimate herbage DMI.

Mixed ration offered and refused groupal were weighed daily and subsamples of offer and residual material were collected and analysed for DM and nutrient components to allow the calculation of mean daily DMI and estimated energy intake by each treatment. In order to compare group *vs* individual DMI of the mixed diet, four cows from each treatment were selected and fed individually during the 12-d period that cows were dosed with alkanes, and, as explained previously, DM offered and refused were registered

Table 1
Mean chemical composition of samples of the mixed diet and herbage hand clipped in both experimental periods.

Composition	Maize-based mixed diet	Sorghum-based mixed diet	Herbage during April	Herbage during June
DM (g/kg)	492 ± 29.8	597 ± 42.9	371 ± 21	279 ± 14
CP (g/ kg DM)	149 ± 23.7	167 ± 18.1	146 ± 7.4	184 ± 13.9
NDF (g/kg DM)	348 ± 41.2	271 ± 21.8	531 ± 3.3	427 ± 3.0
ADF (g/kg DM)	189 ± 31.0	141 ± 16.0	272 ± 6.8	201 ± 2.5
Ash (g/kg DM)	72 ± 8.8	72 ± 0.8	10.2 ± 0.7	11.4 ± 0.5
NEL (MJ/kg DM)[a]	6.82	7.12	6.28	7.12
Herbage mass (DM kg/ha)	–	–	1800 ± 172.8	1834 ± 44.2

[a] Net energy of lactation estimated using the formula (Mieres, 2004):

$$\text{Herbage NEl (MJ/kg DM)} = (2,301 - (0,0289 \times \%\text{ADF}))^{*}4.1868$$

$$\text{Mixed diet NEl (MJ/kg DM)} = (1,909 - (0,015 \times \%\text{ADF}))^{*}4.1868$$

2.6. Animal behaviour

Group and individual grazing behaviour was measured on alternate days throughout weeks 5 and 6 during period 1 (mean 32 ± 11 DIM) and weeks 12 and 13 during period 2 (mean 81 ± 11 DIM). Group grazing behaviour was measured by visual observation where the number of cows eating, ruminating or engaged in other activities whilst at pastureland were recorded every 15 min by 6 observers.

Grazing, ruminating and idling times were recorded using automatic behaviour recorders (SSBR; Rutter et al. 1997) fitted to 6 of the 10 alkane-dosed cows on each treatment. Data was analysed using ‘GRAZE’ software (Rutter et al. 1997).

2.7. Weather

Weather data was collected at the Meteorological Station of EEMAC where daily minimum, maximum and mean temperatures (°C), and precipitation (mm), were recorded throughout the experiment.

2.8. Statistical analyses

Yields of milk, milk fat, milk protein, milk energy output, BCS and LW were analysed with a mixed model with repeated measures in time. All the statistical analysis were performed using the GLIMMIX procedure of SAS User's Guide (2010) except otherwise indicated. The model included the fixed effects of treatment, week, treatment by week interaction and the residual error. The DIM was included as co-variable and block was treated as a random effect. The experimental unit was the cow with 12, 15 and 14 cows used in G0, G1 and G2 treatments, respectively.

The probability of cows grazing, ruminating or engaged in other activities was calculated using a mixed model which included the fixed effects of treatments, hour, their interaction and the residual error. The experimental unit was the cows group and replications were provided by repeated measurements in time (6 replicas in period 1 and 5 replicas in period 2).

The model to analyse group DMI of the mixed ration included the fixed effect of treatment and a residual error term. The experimental unit was the cows group and replications were provided by repeat measures in time (12 replicas in period 1 and 12 replicas in period 2). Analysis of individual DMI of the mixed diet (4 cows per treatment) was conducted using a mixed model with repeated measurements in time including the fixed effects of treatment, day, treatment by day interaction and the residual error. The same model was used to analyse the effect of treatments on grazing time. Herbage DMI was analysed using GLM procedure of SAS User's Guide (2010).

Least square means were separated using Tukey–Kramer tests and significant difference was accepted if $P \leq 0.05$ and tendencies to significance was accepted if $0.05 < P \leq 0.10$.

3. Results

Results of the laboratory analyses of samples of the mixed diet and herbage cut (to represent that eaten by the cows) are presented in Table 1, including estimates of NEL (MJ/d).

3.1. Period 1, weeks 1–10

3.1.1. Intake of mixed diet

DM intakes of the mixed diet on treatments G1 and G2 were significantly reduced compared with those achieved on the *ad libitum* treatment G0 (Table 2). These mean values determined from diet intakes measured with the group-fed animals were within ±0.6 kg of DM of the mean daily DMI determined individually using 4 cows per treatment (25.5, 15.1 and 13.7 kg/cow for treatments G0, G1 and G2, respectively, $P < .0001$).

3.1.2. Herbage intake and grazing behaviour

Behaviour recordings collected from the six cows on each treatment showed that extending the period of access to pasture allowed cows on G2 to significantly increase their total grazing time by 50 min (Table 3), thereby allowing them to significantly increase herbage DMI (Table 2), compared with the cows on G1. Cows on both treatments showed a different grazing pattern, were cows on G2 spent during morning period 46.3 min less grazing but with a higher total grazing time compared with cows on G1 (Table 3). Cows on G2 treatment had an intake rate (kg herbage DM/h) of 1.6 kg/h while cows on G1 showed an intake rate of 1.4 kg/h.

The differences in total grazing times were reflected in the data collected during the common 6- h morning period at pasture by the observers. Overall, during that time, a lower proportion of cows on G2 than on G1 were engaged in grazing (0.34 *vs* 0.68 ± 0.05, respectively, $P=0.004$) and a greater proportion were engaged in ruminating (0.36 *vs* 0.18 ± 0.03, respectively, $P=0.01$, Fig. 2). The difference between G1 and G2 in the proportion of cows grazing and ruminating was greatest during the second hour at pasture during the am session. During the afternoon session, a higher proportion of cows on G2 grazed in each hour (0.78 ± 0.07), than at any other time during the morning grazing session.

Table 2
Mean daily DM intakes by dairy cows of a mixed silage-based diet and grazed herbage, measured during weeks 4 and 5 of lactation (period 1) when offered a mixed diet either *ad libitum* (G0), or at a restricted level (50% of *ad libitum*) with access to pasture for 6 (G1) or 9 h (G2) daily.

	Treatments			S.E. of means	*P*-value
	G0	G1	G2		
Dry matter intake (kg)					
Mixed diet	26.1[a]	14.5[b]	14.3[b]	0.33	< 0.001
Herbage	–	5.5[b]	7.5[a]	0.67	0.049

[a,b,c]Mean values within rows not bearing a common superscript differ significantly ($P < 0.05$)

Table 3
Mean grazing times (min) of dairy cows allowed access to pasture for 6 (G1) or 9 h (G2) daily, in addition to a restricted ration (approx. 50% of *ad libitum* intake) of a mixed diet. Period 1.

	Treatments		S.E. of means	*P*-value
	G1	G2		
Time spent grazing (min)				
Total	231.3	281.3	7.6	< 0.001
During first hour	55.1	54.6	1.7	0.85
During first 3 h	135.5	106.3	4.4	< 0.001
During first 6 h	231.3	185.0	6.8	< 0.001

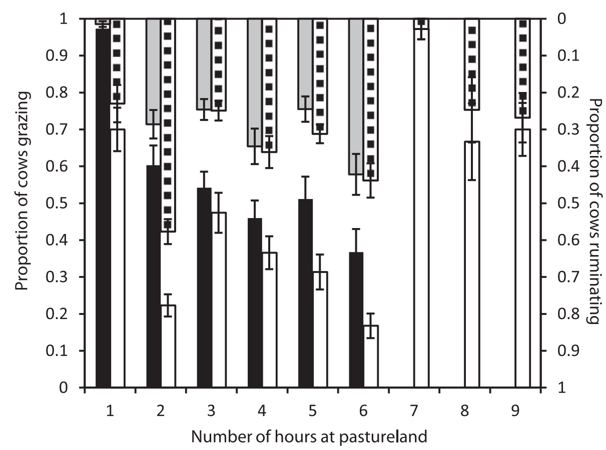

Fig. 2. Proportion of cows grazing and ruminating each hour after the beginning of the grazing session. Grazing (■) and ruminating (▒) activity by cows given access to pastureland for 6 h during the morning (G1 treatment), and grazing (□) and ruminating (▚) activity by cows given access for 6 h during the morning followed by a further period of 3 h during the afternoon (G2 treatment).

3.1.3. Milk production and composition, live weight and body condition score

Results of the analyses of treatment effects on milk yields and milk composition during the first 10-week period are shown in Table 4. Cows on G0 produced significantly more milk (3.5 L/day) than cows on G1, which whilst having slightly lower concentrations of protein and lactose, resulted in significantly greater yields of protein and lactose (0.2 and 0.2 kg/day, respectively). Cows on G0 also produced more milk (3.3 L/day) than those on G2, but with no significant increase in the yields of fat and lactose.

Yields of milk, fat, protein, lactose and output of energy in milk did not differ between cows provided 6 or 9 h access to the pasture (Table 4). Neither the mean LW nor mean BCS differed significantly between cows on G0 and G1. Comparison of mean BCS in cows on G0 and G2 showed an almost significant difference ($P=0.099$) and an interaction among treatment and week was detected ($P=0.01$).

Table 4
Mean daily milk yield, milk composition, estimated NE Milk, live weight and body condition score (BCS) during the first 10 weeks of lactation (period 1) by cows offered a mixed diet either *ad libitum* (G0), or at a restricted level (50% of *ad libitum*) with access to pasture for 6 (G1) or 9 h (G2) daily.

	Treatments			S.E. of means	*P*-value	
	G0	G1	G2		Strategy	Strategy × week interaction
Milk yield (L/d)	37.2^{a}	33.7^{b}	33.9^{b}	0.92	< .001	0.13
Milk component yield (kg/d)						
Fat	1.3	1.3	1.3	0.05	0.15	0.66
Protein	1.3^{a}	1.1^{b}	1.1^{b}	0.04	0.01	0.14
Lactose	1.8^{a}	1.6 ^{b}	1.7^{ab}	0.06	0.01	0.25
Energy (MJ/d)	109.7^{a}	100.5^{b}	105.5^{b}	3.35	0.03	0.30
Milk composition (%)						
Fat	3.7^{b,y}	3.9^{ab,x}	3.9^{a}	0.09	0.03	0.49
Protein	3.3	3.5	3.4	0.06	0.12	0.58
Lactose	4.9^{b}	4.9^{ab}	5.0^{a}	0.04	0.05	0.11
Live weight (kg) and Body condition score (1–5 point scale)						
Live weight	638	635	645	9.0	0.62	0.55
BCS	2.86^{x}	2.78^{xy}	2.71$^{y.}$	0.05	0.12	0.01

Mean values within rows not bearing a common superscript (^{a,b,c}) differ significantly ($P<0.05$) (^{x,y,z}) show a trend ($0.05<P<0.1$).

Table 5
Intakes of a mixed diet and herbage estimated during period 2 (weeks 12 and 13 of lactation) by dairy cows provided access to pasture for 6 h during the morning. During the first 10 weeks of lactation cows had received either the mixed diet *ad libitum* without grazing (Post-TMR) or a restricted ration of the mixed diet with access to pasture for 6 h daily (G1).

	Treatments		S.E. of means	*P*-value
	PosT-TMR	G1		
Dry matter intake (kg)				
Mixed ration	20.3	19.8	0.20	0.11
Herbage	5.4	6.5	0.68	0.26

3.2. Period 2, weeks 11–13

3.2.1. Intake of mixed diet

There was no significant carryover effect of previous feeding strategy on intake of the mixed diet by cows on the Post-TMR and G1 treatments, refusals being few (Table 5). These mean values determined from diet intakes measured with the group-fed animals were within ±0.05 kg of DM of the overall mean daily DMI determined individually using 4 cows per treatment (20.3 and 19.8 for Post-TMR and G1, respectively, $P=0.12$).

3.2.2. Grazing behaviour and herbage intake

Analysis of the automatic behaviour recordings showed no significant treatment effect on total grazing time (217±15.4 min, $P=0.89$), grazing time during the first hour at pasture (45±8.2 min, $P=0.21$) and grazing time during the first 3 h at pasture (83±6.8 min, $P=0.37$).

There were no significant treatment effects on group grazing behaviour with the proportion of cows engaged in grazing being 0.5±0.06, and in ruminating being 0.3±0.04 ($P=0.75$). The proportion of cows engaged in grazing and ruminating within each hour is shown in Fig. 3.

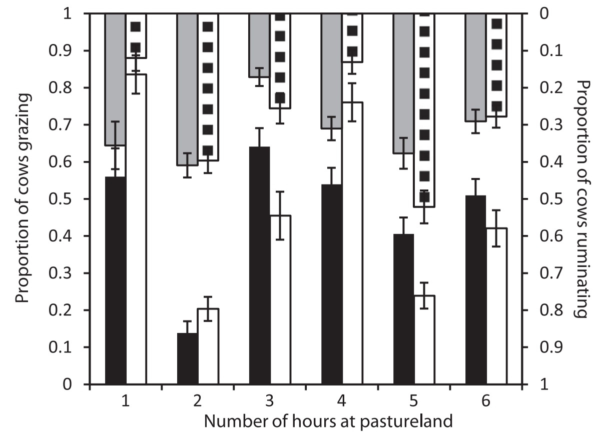

Fig. 3. Proportion of cows grazing and ruminating each hour after the beginning of the grazing session. Grazing (□) and ruminating (■) activity by cows given access to pastureland for 6 h during the morning following an approximately 10-week period during which they had received a total mixed ration *ad libitum* (Post-TMR), and grazing (■) and ruminating (■) activity by cows given access to pastureland for 6 h during the morning during all the experimental period (G1 treatment).

3.2.3. Milk production and composition, live weight and body condition score

Results of the analyses of treatment effects on milk yields and milk composition during weeks 11–13 are shown in Table 6, together with those of live weight and BCS. There were no significant treatment effects on milk yield or composition, and therefore no effects on yields of milk fat, protein, lactose or energy (Table 6). Treatments did not affect BW or BCS.

3.3. Weather data

Total rainfall during March, April, May and June was 55, 149, 132 and 117 mm, respectively, compared with the previous 30-y means of 147, 103, 77 and 70 mm (National Weather Direction, period 1961–1990). Mean monthly temperatures for the four months were 22.2, 14.4, 14.9 and 11.8 °C, respectively.

4. Discussion

4.1. Period 1, weeks 1–10

4.1.1. Herbage and total DMI

The different feeding strategies resulted on differences on total DMI as G0 achieved higher DMI and daily NEl (MJ/d) consumption than G1 and G2 treatments (Table 2), consistent with similar studies in mid lactation (Bargo et al., 2002; Vibart et al., 2008). Vibart et al. (2008) discussed that reducing the proportion of TMR in diets increased herbage consumption but reduced total DMI on grazing strategies. Authors argue that those differences would be related to restrictions on time spent grazing and the use of grazing windows that do not respect cows diurnal grazing patterns.

The effect of different grazing windows and hours of access to paddock tested in the present study resulted in higher intake of herbage DM on cows with 9 h of access to the paddock distributed during the morning and evening (G2 *vs* G1, Fig. 1). G2 cows ate 2 more kg of herbage than G1 cows, with similar intake of mixed ration, leading to a higher intake of NEL (+11.7 MJ/d). This result is consistent with previous reports (Soriano et al., 2001; Bargo et al., 2002; Vibart et al., 2008), where cows offered pasture did not seem to substitute pasture for ration, as very low refusals of the ration were registered in both grazing strategies. Also, Soriano et al. (2001) found that cows grazing after pm milking achieve

Table 6
Mean daily milk yield, milk composition, estimated NE Milk, live weight and body condition score (BCS) during weeks 11–13 of lactation by dairy cows provided access to pasture for 6 h during the morning. During the first 10 weeks of lactation cows had received either the mixed diet *ad libitum* without grazing (Post-TMR) or a restricted ration of the mixed diet with access to pasture for 6 h daily (G1).

	Treatments		S.E.	*P*-value
	Post-TMR	G1		
Milk yield (L/d)	37.2	36.3	1.10	0.26
Milk component yield (kg/d)				
Fat	1.3	1.3	0.05	0.71
Protein	1.2	1.2	0.05	0.93
Lactose	1.8	1.8	0.08	0.92
Energy (MJ/d)	108.4	108.9	4.19	0.96
Milk composition (%)				
Fat	3.5	3.6	0.12	0.37
Protein	3.3	3.4	0.06	0.62
Lactose	5.0	5.0	0.05	0.76
Live weight (kg) and Body condition score (1–5 point scale)				
Live weight (kg)	619	631	19.2	0.67
BCS	2.8	2.6	0.10	0.19

higher herbage DMI than after am milking in spite of having the same number of hours of access to the paddock.

4.1.2. Grazing behaviour

Access time to the paddock had an effect on grazing behaviour, as G1 cows spent 84% of time grazing *vs* 67% for G2 cows, at the expense of a shorter rumination time for G1 cows during the morning session (Fig. 2). This behaviour pattern is similar to that reported by Chilibroste et al. (2007) and Pérez-Ramirez et al. (2009), where reductions in access time to pastureland resulted in increases in the proportion of time spent grazing, and reductions in the proportion of time spent ruminating. Daily grazing patterns showed that G2 cows grazed more intensively during the afternoon than in the morning, which is in agreement with Gibb et al. (1998) where cows grazing in the afternoon maximised DMI with extended grazing sessions at a higher intake rate than cows grazing in the morning.

Cows on G2 treatment which had three extra hours of access to the paddock, grazed 50 min longer than G1 achieving a higher herbage DMI, which could have allowed a greater milk production. However, since G2 cows had extra maintenance requirements and a greater BCS mobilisation, the absorbed nutrients might have been used in different functions instead of milk synthesis.

4.1.3. Milk production and composition

The effect of feeding 100% mixed diet *vs* 6 or 9 h at pasture plus mixed diet on milk production, fat content and protein milk yield were consistent with similar studies on early (Acosta et al., 2010; Sprunck et al., 2012) and mid lactation (Bargo et al., 2002; Vibart et al., 2008). Those differences could be explained by differences in total DMI observed and requirements of NE of maintenance between not grazing and grazing cows, reported previously by Bargo et al. (2002). However, G0 resulted superior to G1 treatment on milk energy output (MJ/d) with no differences in BCS, whereas, when compared to G2, no differences were found on milk energy output (between G0 and G2) with a trend to differences in BCS ($P=0.099$, Table 4). Vibart et al. (2008) found similar results, where cows fed grazing plus TMR produced similar 4% fat corrected milk (FCM) to cows fed 100% TMR, attributed to higher conversion efficiency on grazing *vs* TMR strategies (0.68 *vs* 0.80 kg feed/kg FCM 4%, respectively). In our study grazing treatments also achieve higher conversion efficiency (0.62, 0.65 *vs* 0.78 kg feed/kg FCM 4% for G1, G2 and G0 treatments, respectively).

The lack of differences on fat production differs from previous reports (Bargo et al., 2002; Acosta et al., 2010; Sprunck et al., 2012) and was due to the lower fat content in milk for G0 cows. Those results were probably related to the lower NDF content of the G0 diet (210 *vs* 294 g NDF/kg DM of silage+pasture, for G0 and G1, respectively), plus a possible deficit of insoluble CP detected by SHIELD program (2013), analysis that predicted a decrease on the fibre degradability affecting availability of precursors for milk fat and milk protein synthesis. Moreover, the insoluble CP deficit could also explain the lack of differences in milk protein content expected according to previous research (Bargo et al., 2002).

In spite of the three extra hours of access to the paddock and the two extra kg of herbage DMI taken by G2 cows, no differences were found in milk production or composition between G1 and G2 treatments (Table 4). This would be likely because G2 cows walked 3.4 km/d more and graze 50 min extra than G1 cows. According to the NRC (2001), requirements of NEl (MJ/d) for G2 cows would have been ~5.0 MJ/d higher than G1, difference which could correspond to the extra energy obtained from the extra herbage DM intake.

4.2. Period 2, weeks 11–13

4.2.1. Grazing behaviour

From 61 to 90 DIM, grazing behaviour of Post-TMR and G1 cows was monitored as an adaptation mechanism of G0 cows to a grazing feeding strategy. In contrast to our hypothesis, there were no differences on grazing behaviour between treatments. An interaction between treatment and time was detected (Fig. 3) due to the lower grazing activity on Post-TMR during the last two hours in the pasture but with no effects on other variables.

4.2.2. Production responses

During the second period of the experiment there were no differences in milk production or milk composition between Post-TMR and G1 cows, with Post-TMR cows maintaining their production despite the change of diet while G1 cows increased their production (Table 6). This could be related to a better quality of pasture in period 2 than in period 1 (June *vs* April, Table 1) related to better weather conditions in period 2, and an increase of mixed ration and herbage DMI. Therefore, BCS and LW of cows on different treatments in period 1 were not different in period 2. This suggests that the use of herbage to replace mixed rations (to 25%) in mid lactation allows high levels of milk and solids production, despite feeding strategy differences during the first 60 DIM.

5. Conclusions

As hypothesised, cows fed 100% mixed diet during the first 10-wk of lactation had a higher DMI, milk and protein production when compared with cows with 6 or 9 h access to pastureland plus mixed diet. However, milk fat production and LW were not different between treatments and only a tendency for a higher BCS was found between G0 and G2 cows. On feeding strategies including grazing during the first 10-wk of lactation the larger daily access time to pastureland improved herbage DMI, although with no productive consequences, likely due to differences in maintenance requirements on cows that walk twice a day to the paddock. The cows subjected to a change of feeding system from 100% mixed diet to 6 h access to pastureland plus a mixed diet, adapted immediately to the new diet and sustained their production. In conclusion, 100% mixed diet systems had higher milk production than grazing systems in early lactation but differences could be shortened with increases in access time to pastureland. Results from this study show that the use of herbage to replace mixed diets (to 25%) will maintain high milk and solids on milk production after the first 65 DIM.

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. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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