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ADAPTACIÓN DE VACAS LECHERAS A ESTRATEGIAS INTEGRADAS DE MANEJO DEL PASTOREO Y SUPLEMENTACIÓN

Conducta en pastoreo, fermentación ruminal y producción

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RESUMEN

El objetivo del proyecto fue identificar estrategias de alimentación en pastoreo que mejoren la eficiencia de uso de los recursos alimenticios, especialmente la pastura. La investigación involucró 4 experimentos donde se estudió el efecto del tiempo y momento de acceso a la pastura y el momento de la suplementación con ensilaje de maíz sobre variables productiva y de fermentación ruminal. La producción de leche y el consumo de MS de forraje (**CF**) fue mayor para las vacas que pastorearon 8 h respecto a 4 h, y no hubo diferencias para el momento de acceso. Aunque las vacas que pastorearon de 11-15 h (T11-15) dedicaron menos tiempo al pastoreo, con mayor peso de bocado y mayor tasa de consumo que cuando pastorearon de 7-11 h (T711), lo que explica la mayor producción de proteína láctea. El pH ruminal en T11-15 cayó más rápido que T7-11 en línea con las mayores concentraciones de AGV y amonio observadas en T11-15. La concentración y producción de grasa láctea fue mayor en las vacas en que el ensilaje de maíz se distribuyó en dos veces a que cuando se suministró en la mañana consistente con los CF observados. El momento de suplementación con ensilaje de maíz afectó la cinética ruminal en términos de pH, concentración de amonio, AGV y fracciones líquido/sólido. El ensilaje de maíz previo al pastoreo actuó como amortiguador de la fermentación ruminal y al mismo tiempo como una señal de saciedad e impactó en el llenado y fermentación ruminal a través de los cambios en los patrones de pastoreo y rumia y el consumo total de MS. La investigación permitió comprender como las vacas integran los procesos de ingestión-digestión en un espacio temporal diario con una cantidad de alimentos finita, mostrando una gran plasticidad y capacidad de adaptación. Estos resultados, permiten mejorar la eficiencia de utilización de los recursos, a través del diseño de estrategias de alimentación alternativas.

Palabras claves: consumo, pastoreo, comportamiento ingestivo, fermentación ruminal

DAIRY COWS ADAPTATION TO INTEGRATED STRATEGIES OF GRAZING AND SUPPLEMENTATION MANAGEMENT, GRAZING BEHAVIOUR, RUMINAL FERMENTATION AND PRODUCTION

SUMMARY

The objective of the project was to identify feeding strategies under grazing, to improve the efficiency of the use of feeding resources, especially the pasture. The research strategy involved 4 experiments, where effect of access time to pasture and time of grazing allocation and timing of corn silage supplementation on productive and ruminal fermentation variables was studied. Milk yield and herbage dry matter intake (HDMI) was higher for cows that grazed 8 h compared to 4 h, and there were no differences for the time of grazing allocation. However, cows that grazed from 11-15 h (T11-15) spent less time grazing with a greater bite mass and intake rate than cows that grazed from 7-11 h, and could explain the higher milk protein production. Ruminal pH of T11-15 dropped faster than T7-11 cows, which was in line with the higher VFA and ammonia concentrations observed in T11-15. Milk fat concentration and yield were higher in cows that received corn silage divided twice a day than those who received it at one time consistent with the higher HDMI observed. The timing of corn silage supplementation related to grazing session affected ruminal kinetics in terms of pH, ammonium and VFA concentration, and liquid/solid ratio fractions. Corn silage before grazing worked as a buffer for ruminal fermentation, but at the same time as a satiety signal and impact on rumen fill and fermentation due to changes in grazing and ruminating pattern and total DMI. The research strategy allowed us to understand how the grazing dairy cows modify ingestion and digestion on a daily basis, when they are exposed to a limited amount of feeding resources in different setups, showing high plasticity and adaptation on both: ingestion and digestion. A new window of opportunities has been opened after these results, to improve efficiency of the use of feeding resources applying alternative feeding strategies.

Keywords: intake, grazing, ingestive behaviour, ruminal fermentation

1. INTRODUCCIÓN

Uruguay como país netamente exportador de lácteos debe integrar en sus modelos productivos aspectos relacionados a la cantidad y calidad de productos, el equilibrio con el medio ambiente y con procesos productivos que den seguridad a los consumidores y a la sociedad en general. No obstante lo anterior, debe cuidar muy especialmente los costos de producción, ya que son centrales en la competitividad de los sistemas lecheros y del sector en su conjunto. En este sentido, los sistemas de producción en Uruguay con mejores resultados económicos, son los que mantienen alto nivel de participación del forraje (cosecha directa y reservas) en la dieta de los animales (Chilibroste et al., 2011) y relativamente bajos costos de producción comparados con el resto del mundo (IFCN, 2016).

La pastura en el período otoño/invierno es una limitante en los sistemas de producción de leche, como consecuencia de moderadas tasas de crecimiento, reducción del área efectiva de pastoreo y el aumento en la carga animal (DIEA, 2010, Chilibroste et al., 2002). Esto resulta en una menor participación de la pastura cosechada en forma directa en las dietas y da lugar al uso de concentrados y reservas como suplementos para cumplir con los requerimientos de producción en este período. Por esta razón y por ser el forraje un insumo de alto impacto económico, es muy relevante usarlo en forma eficiente; a través del manejo del pastoreo y/o de la suplementación, donde el momento de suministro puede ser una alternativa para mejorar tanto el consumo total de materia seca (**CMS**) como la eficiencia en el uso de los nutrientes.

El proceso de intensificación de los sistemas de producción respecto al uso de los recursos ha seguido trayectorias diversas. La principal estrategia de intensificación en Uruguay se basó en: un aumento de la productividad a través del aumento de la carga animal como producto de una reducción del área lechera (DIEA, 2017) y niveles crecientes de suplementación (concentrados y reservas) y en particular a través de cambios en la cantidad y composición de los concentrados (Chilibroste, 2015).

La producción de leche por vaca está explicada principalmente por el CMS y en menor medida por la eficiencia con que la dieta es digerida y se absorben los nutrientes. En condiciones pastoriles para alcanzar el CMS que una vaca lechera de alto potencial requiere, es necesario contar con muy buenas condiciones de masa, altura y asignación de forraje y controlar el manejo de los animales en términos de rutina, suficiente tiempo de acceso a la pastura, tiempos de descanso en condiciones de confort, etc. (Chilibroste et al., 2005). Por lo tanto, la restricción de acceso al pastoreo puede afectar el CMS (Chilibroste et al., 2012, Pérez-Ramírez et al., 2008) pero esto depende de la severidad de la restricción y de las posibilidades que tengan los animales de incrementar su tasa de consumo (Chilibroste et al., 2015, Soca et al., 1999). Los factores que afectan la tasa de consumo en pastoreo han sido ampliamente estudiadas, principalmente en una escala de corto plazo, como la estructura de la pastura (Gibb, 2006, Orr et al., 2004), el estado fisiológico, requerimientos nutricionales y duración del ayuno (Chilibroste et al., 2015, Gibb, 2006, Patterson et al., 1998).

Si bien los rumiantes tienen un patrón de actividades de pastoreo, rumia y descanso definido a lo largo del día, el manejo del pastoreo, las características de la pastura y la forma en que el pastoreo es combinado con otros alimentos podrían modificar estos patrones (Gibb et al., 1998, Orr et al., 1997, Hodgson, 1985). Asimismo, la combinación de estos factores podría afectar el aporte de nutrientes y su posterior utilización (Orr et al., 2004, Delagarde et al., 2000, Gibb et al., 1998).

En los sistemas pastoriles donde la pastura es el componente principal de la base alimenticia, la manipulación de los nutrientes disponibles para el rumiante, basada en el control del proceso de pastoreo, es una alternativa para lograr cambios del producto sin modificar en forma importante los costos de producción. El control del proceso de pastoreo involucra decisiones relacionadas al manejo de los animales, de la alimentación y manejo de la pastura como el principal alimento, entre otros. Las condiciones de manejo del pastoreo así como características de la pastura afectarán la actividad de pastoreo a través del tiempo efectivo de pastoreo, el peso y tasa de bocados que determinan la tasa de consumo y finalmente el CMS (Gibb, 2006).

Los sistemas lecheros de alto rendimiento requieren de un gran aporte y balance de nutrientes, tanto para el animal como para la población microbiana del rumen. Esto lo transforma en un desafío que exige buscar alternativas para mejorar su aprovechamiento. Es en este contexto donde establecer diferentes estrategias, ya sea de acceso al pastoreo o de suplementación con cantidades limitadas de un mismo recurso, son opciones de intervención que pueden tener impacto en los resultados productivos y económico de las diferentes estrategias de alimentación.

Este trabajo tiene por objetivo generar resultados que integren en condiciones de pastoreo directo y con pasturas limitantes, los cambios en el CMS y performance productiva a través de cambios en los patrones de ingestión de los diferentes componentes de la dieta y su posterior utilización a una escala temporal (diaria), mayor a la encontrada en la literatura hasta el momento.

1.1 HIPÓTESIS

1. En condiciones restringidas de alimentación, el control del tiempo de acceso a la pastura y la hora de ingreso al pastoreo afectará la tasa de consumo, el CMS y por tanto los resultados productivos de vacas lecheras.

2. En estas condiciones el momento de la suplementación con ensilaje de maíz respecto a la sesión de pastoreo afectará el patrón de ingestión, lo que debería resultar en diferentes CMS, como resultado del ambiente ruminal y por tanto diferentes aportes de nutrientes para la vaca.

3. La capacidad de adaptación de las vacas lecheras a los cambios en estrategias de alimentación se expresa a través del comportamiento ingestivo.

1.2 OBJETIVO GENERAL

Estudiar las respuestas en comportamiento/conducta animal a diferentes estrategias de manejo del pastoreo y suplementación y sus efectos en los patrones de ingestión y fermentación ruminal, consumo de MS de forraje (CF), CMS y resultados productivos en vacas lecheras.

1.3 OBJETIVOS ESPECÍFICOS

Estudiar y comprender:

1. El efecto del control del tiempo de acceso a la pastura y la hora de ingreso al pastoreo sobre el patrón diario de pastoreo, comportamiento ingestivo y CMS y la producción y composición de leche.

2. El efecto de la hora de ingreso al pastoreo restringido en la fermentación ruminal (pH, ácidos grasos volátiles (**AGV**) y amonio).

3. El efecto del momento de la suplementación con ensilaje de maíz con respecto al pastoreo en el comportamiento ingestivo, CMS, producción de leche y composición en vacas.

4. Evaluar el efecto del momento de la suplementación con ensilaje de maíz respecto al pastoreo: en los diferentes pooles del rumen, la

fermentación ruminal (pH, AGV y amonio) y degradabilidad *in situ* de la MS de vacas lecheras en pastoreo.

1.4 ESTRATEGIA DE LA INVESTIGACIÓN

En base a las hipótesis y para cumplir los objetivos planteados se realizaron 4 experimentos.

Los experimentos 1 y 2 se realizaron en el mismo momento sobre la misma pastura con animales en producción con la particularidad que para el experimento 2 los animales fueron fistulados de rumen y se manejaron en forma individual para que consumieran forraje en áreas pre establecidas. En el **Experimento 1** se evaluó el efecto de la restricción del pastoreo de 8 a 4 h y dentro del tiempo restringido (4 h) dos horarios de ingreso al mismo 7 vs 11 h en el patrón de ingestión, comportamiento, CF y producción y composición. En el **Experimento 2** se evaluó el efecto de la hora de ingreso al pastoreo 7 vs 11 h en el patrón de fermentación ruminal y la concentración de sus productos finales AGV y amonio así como el pH ruminal y sus fluctuaciones.

Los Experimentos 1 y 2 fueron publicados en:

Mattiauda, D.A., Tamminga, S., Gibb, M.J., Soca, P., Bentancur, O., 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. Livest. Sci. 152, 53-62. doi:10.1016/j.livsci.2012.12.010

En el **Experimento 3** se evaluó el efecto del momento de la suplementación con ensilaje de maíz con respecto a la sesión de pastoreo sobre el comportamiento ingestivo, CMS y la producción y composición de la leche en vacas con acceso restringido (6 h) a la pastura.

El experimento 3 fue publicado en:

Mattiauda, D.A., Gibb, M.J., Carriquiry, M., Tamminga, S., Chilibroste, P., 2018. Effect of timing of corn silage supplementation to Holstein dairy cows given limited daily access to pasture: intake and performance. Animal 1-9. doi:10.1017/S1751731118000794

En el **Experimento 4** se evaluó el efecto del momento de la suplementación con ensilaje de maíz con respecto al pastoreo en los pooles para las diferentes fracciones del rumen, pH ruminal y concentraciones de AGV y amonio en una pastura con acceso restringido.

Este experimento fue enviado para publicación:

Effect of timing of corn silage supplementation to Holstein dairy cows given limited daily access to pasture on rumen kinetics and fermentation. Animal (en revisión).

2. ACCESO RESTRINGIDO Y MOMENTO DE INICIO DEL PASTOREO EN VACAS HOLANDO: COMPORTAMIENTO INGESTIVO, CONSUMO Y PRODUCCIÓN DE LECHE[1]

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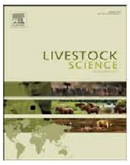

Restricting access time at pasture and time of grazing allocation for Holstein dairy cows: Ingestive behaviour, dry matter intake and milk production

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ABSTRACT

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

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

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

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

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

1. Introduction

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

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

Although IR in grazing dairy cows has been studied comprehensively, most studies are based on short-term observations (e.g., duration of 1 h or less; Hodgson, 1985). Besides the sward characteristics mentioned above, IR is affected by animal characteristics (physiological status, nutritional requirements and appetite) and management (Gibb, 2006; Patterson et al., 1998). The effect of restricting the period of access to pasture on grazing behaviour, daily DMI and productive performance of dairy cows is poorly understood. Restricting the period during which cattle have access to pastures can increase herbage production and utilization by reducing the negative effects of cattle on the sward, such as treading and fouling. Studies with beef cattle, in which the period of access to pasture has been restricted, have shown variable effects on grazing behaviour and performance, depending upon the severity of access restriction and the grazing conditions (Gekara et al., 2005; Smith et al., 2006). A recent study of dairy cows (Perez-Ramirez et al., 2008) showed that restricting access to pasture from 8 to 4 h per day decreased GT by 2 h which, despite large increases in IR and proportion of available time spent grazing, reduced herbage intake and milk production. As far as we are aware, few studies (Chilibroste et al., 2007) with dairy cows have addressed the effect of restricting access time at pasture, whilst maintaining supplementation (silage+concentrates) at a fixed level, on ingestive behaviour, milk production and composition.

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

2. Materials and methods

2.1. Experimental design, animals, and treatments

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

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

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

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

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

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

2.2. *Herbage mass and pasture depletion*

To determine the appropriate paddock sizes, herbage mass was calculated weekly using a double sampling technique adapted from Haydock and Shaw (1975). Every 14 days, three replicate sets of five sampling locations were selected within the areas to be grazed. The five locations were chosen to represent a short, a tall and three areas of intermediate sward height. At each location, sward height was measured to the nearest 0.5 cm using a rising plate metre (RPM, Ashgrove Co., Palmerston North, NZ) and 30 × 30 cm squares of pasture on the same area were cut to ground level with shearing scissors. The cut herbage was collected, weighed, and sampled for determination of DM content to calculate herbage DM mass and derive a linear regression relating sward height (RPM). Each week, herbage mass was calculated by measuring sward height with the RPM at 20 points within the paddocks and applying the regression determined the current or previous week.

Table 1
Chemical composition of supplements.

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

[a] Estimated from equation of National Research Council (2001).

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

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

2.3. *Herbage DM intake*

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

2.4. *Grazing behaviour*

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

Table 2
Chemical composition of allowed herbage mass by treatments.

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

[a] (T7–15) grazing between 07.00 and 15.00 h.
[b] (T7–11) grazing between 07.00 and 11.00 h.
[c] (T11–15) grazing between 11.00 and 15.00 h.

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

2.5. *Chemical composition*

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

2.6. *Rumen fermentation study*

Simultaneously and adjacent to the previous experimental procedures, six rumen-cannulated primiparous lactating cows yielding 19.5 ± 4.58 kg milk/day, at 68 ± 7.4 days in milk and 448 ± 19.0 kg LW, were blocked by milk yield and days in milk and randomly assigned to T7–11 and T11–15 treatments. Cows grazed individually, tethered within a circular plot as described by Chilibroste et al. (2000). The mean plot size was of approximately 115 m^2/cow/d aimed to achieve an herbage allowance of 18 kg/DM/cow/d. Each time there was a variation in herbage mass the individual plot area was adjusted as appropriate. Herbage mass was measured by the same method as described for the main experiment. Feeding and milking management were also the same for both studies which ran simultaneously during 6 weeks. From wk 1–6, one day per wk, rumen fluid was collected from cows on T7–11 and T11–15 at 0, 1, 2, 4, 8, 10, 11, 12, 14, 18, and 22 h and 0, 1, 2, 4, 6, 7, 8, 10, 14, 18, and 22 h, respectively, (0 = beginning of grazing), to determine pH, ammonia and VFA concentrations. Rumen samples were filtered through a cheese cloth and an aliquot sample was taken immediately to measure pH with a mobile pH-metre (Oakton, Eutech Instruments, Malasia). Aliquots samples acidified in ratio of 20:1 relation, with sulphuric (95.6%) and orthophosphoric (85%) acids were collected and frozen until analysis of ammonia and VFA contents, respectively. Ammonia was determined by distillation with MgO (Bremner, 1960) and VFA by gas-chromatography (Chilibroste et al., 2000).

2.7. *Calculations and statistical analysis*

Net energy for lactation (NE_L) was calculated as described by National Research Council (2001). Milk energy output was calculated weekly as NE_L = milk yield × [(0.0929 × fat%) + (0.0563 × true protein%) + (0.0395 × lactose%)], using milk composition data derived weekly from analysis of the four consecutive samples (National Research Council (2001)).

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

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

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

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

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

3. Results

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

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

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

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

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

[a] (T7–15) grazing between 07.00 and 15.00 h.
[b] (T7–11) grazing between 07.00 and 11.00 h.
[c] (T11–15) grazing between 11.00 and 15.00 h.
[d] RPM = rising plate metre.

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

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

[a] (T7–15) between 07.00 and 15.00 h; 8 h.
[b] (T7–11) between 07.00 and 11.00 h or (T11–15) between 11.00 and 15.00 h, 4 h.

Table 5
Daily dry matter intake of herbage, maize silage and concentrate by dairy cows allowed access to pasture for 8 h at 07.00 h (T7–15) or for 4 h, commencing at 07.00 h (T7–11) or at 11.00 h (T11–15).

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

[a] (T7–15) between 07.00 and 15.00 h; 8 h.
[b] (T7–11) between 07.00 and 11.00 h or (T11–15) between 11.00 and 15.00 h, 4 h.

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

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

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

3.2. *Dry matter intake and pasture depletion*

Daily DM intakes of dietary ingredients are presented in Table 5. Allowing cows access to pasture for 8 h, compared with 4 h, significantly increased their estimated intake of herbage DMI. However, estimated herbage DMI did not differ between cows allowed access either early of late in the morning. Intakes of maize silage and concentrate DM did not differ significantly between treatments. Daily intakes of total and digestible DM were significantly greater in cows offered 8 h access compared with 4 h access. The same pattern was observed when herbage and total DMI were analyzed relative to cow LW.

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

3.3. Grazing behaviour

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

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

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

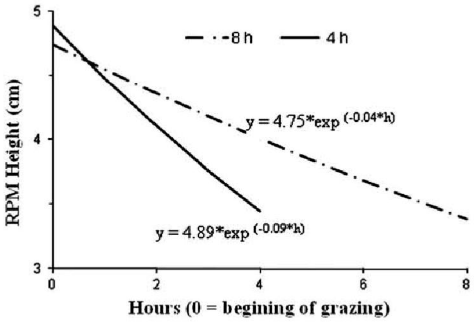

Fig. 1. Regressions representing pasture depletion measured at hourly intervals during grazing sessions lasting 8 h (T7–15, – ● –) or 4 h (joint line for T7–11 and T11–15, –).

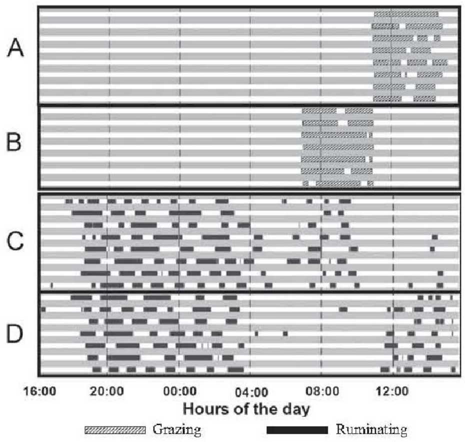

Fig. 2. Temporal patterns of grazing (A, B) and ruminating (C, D) activity by dairy cows allowed access to strips of pasture between 11.00 and 15.00 h (A, C) or between 07.00 and 11.00 h (B, D).

Table 6
Mean time spent grazing, ruminating or idling, number of biting, non-biting and total grazing jaw movements over 24 h, and derived estimates of intake rate and bite mass by dairy cows allowed access to pasture for 4 h, commencing at 07.00 h (T7–11) or at 11.00 h (T11–15).

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

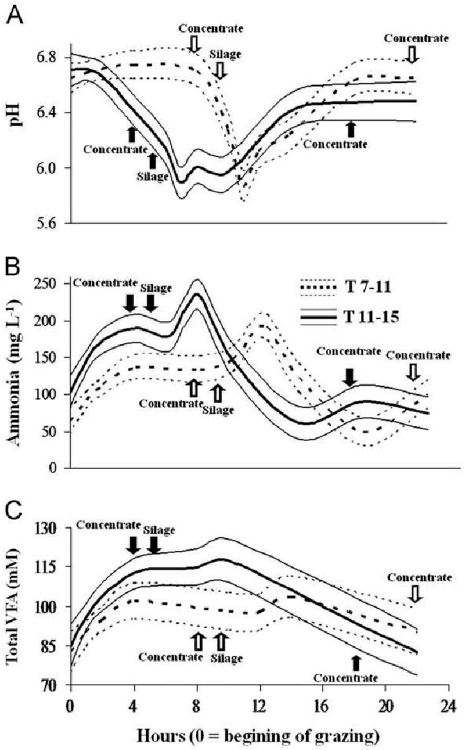

Fig. 3. Diurnal patterns of ruminal pH (A), ammonia (B) and VFA (C) concentrations of strip-grazed dairy cows with different timing of grazing allocation early in the morning (—T7–11) or late in the morning (T11–15).

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

3.4. *Rumen fermentation*

The temporal patterns of rumen pH, ammonia and VFA concentrations, adjusted to the beginning of the grazing session (0 h), are presented in Fig. 3. Rumen pH was significantly affected by treatment. Cows on T11–15 showed a decline in pH 2 h after the start of grazing, even before the intake of supplements, and reached the minimum value 5 h later (7 h from beginning of grazing session). In contrast, cows on T7–11 exhibited a delay of almost 8 h before pH declined following intake of supplement, achieving the minimum pH value 11 h after the start of the grazing session. Although minimum values of pH did not differ between treatments (pH 5.9), pH remained low for a longer time in T11–15 than in T7–11 cows. After 12 h there were no differences in rumen pH values between treatments and the highest values (pH 6.6) were reached around 16 and 18 h after the beginning of their grazing sessions.

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

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

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

4. Discussion

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

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

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

Kristensen et al. (2007) reported a decrease in daily herbage DMI of 2.3 kg (18.1%) when dairy cows were restricted to 4 h compared to 9 h access to pasture. In their study herbage DMI was estimated by the difference between energy requirements for milk production and intake of metabolizable energy (ME) in supplemental feeds, divided by the ME concentration of the hand plucked herbage samples. In the present study cows with a restricted access time to pasture probably increased IR as a behavioural response to the time restriction. However, the potential higher IR of restricted cows did not fully compensate herbage DMI that was higher for 8 than for 4 h access time (Chilibroste et al., 2007).

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

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

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

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

Grazing behaviour was only determined in the 4 h treatment groups. The reduced grazing time found in T11–15 cows was associated with fewer bites and non-biting grazing jaw movements. However, herbage DMI did not differ significantly between the two treatments, which suggests that without any significant difference in bite rate, cows on T11–15 were able to achieve a greater bite mass and higher short-term intake rate. Such a proposition is supported by results of other studies. For example Gibb et al. (1998) demonstrated that, under relatively constant sward conditions achieved by variable continuous stocking management, dairy cows increased their bite mass and short-term DM intake rate as the day progressed. Similar results were also reported by Orr et al. (1997). In addition to such increases in short-term intake rate over the course of the day, the chemical composition of the herbage changes, with increases in DM and soluble carbohydrate contents in the afternoon having also been associated with greater herbage DMI later in the day (Orr et al., 2001; Delagarde et al., 2000). This has been interpreted to be an optimum foraging strategy to harvest herbage of higher digestibility, with higher concentrations of soluble carbohydrates and DM (Gibb et al., 1998; Taweel et al., 2004). The tendency for a higher short-term IR in T11–15 cows who spent less time and probably less energy to achieve the same DMI as T7–11 cows, may be related to the greater milk protein yield observed in T11–15 treatment. The IR of T11–15 cows could be caused by the greater fasting time of T11–15 cows as reported before (Chilibroste et al., 2007; Gregorini et al., 2008; Patterson et al., 1998). It is also known that cattle adapt their grazing behaviour in anticipation of future events, including energy requirements, and so can be hyperphagic under certain conditions (Baile and McLaughlin, 1987; Provenza, 1995).

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

5. Conclusions

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

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

Conflict of interest statement

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

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3. EFECTO DEL MOMENTO DE LA SUPLEMENTACIÓN CON ENSILAJE DE MAÍZ A VACAS LECHERAS HOLANDO CON ACCESO RESTRINGIDO AL PASTOREO CONSUMO Y RESULTADOS PRODUCTIVOS[2]

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

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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.

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).

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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 × [(0.0929 × fat %) + (0.0563× true protein %) + (0.0395 × 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 × 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 × 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 × 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 × 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 $P < 0.10$.

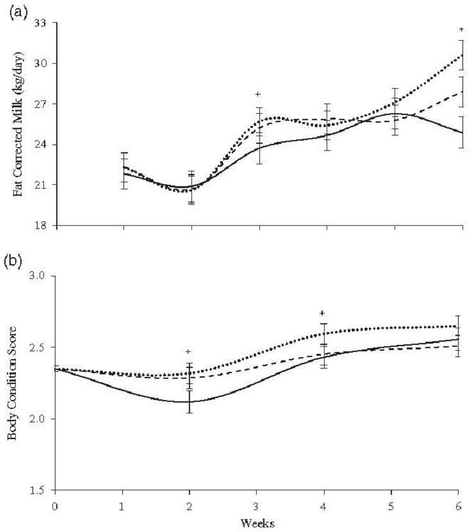

Figure 1 Milk yield 4% fat-corrected (a), and body condition score (b) of strip-grazed dairy cows offered corn silage at 0800 h (AM, ——), equally offered at 0800 and 1700 h (AM-PM, ……) or at 1700 h (PM, - - - -). Within a week, *indicates the difference ($P < 0.05$), + indicates a tendency to be different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

× 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 $\geqslant$ 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 $y = 31.52\exp^{(-0.01t)}$ ($R^2 = 0.77$), 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 $P < 0.10$.

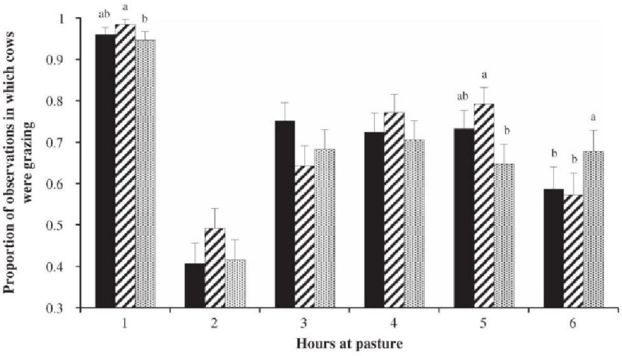

Figure 2 The proportion of visual observations in each hour at pasture during which cows were grazing, when offered corn silage either in a single meal before (AM, ■), in two equal meals before and after (AM-PM, ▨) or in a single meal (PM, ▦) after 6 h at pasture. ^{a,b}Columns within an hour with different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

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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.

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4. EFECTO DEL MOMENTO DE LA SUPLEMENTACIÓN CON ENSILAJE DE MAÍZ A VACAS LECHERAS HOLANDO CON ACCESO RESTRINGIDO AL PASTOREO EN LA CINÉTICA Y FERMENTACIÓN RUMINAL[3]

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[3] Animal (en revisión)

4.1 EFFECT OF TIMING OF CORN SILAGE SUPPLEMENTATION TO HOLSTEIN DAIRY COWS GIVEN LIMITED DAILY ACCESS TO PASTURE ON RUMEN KINETICS

D. A. Mattiauda[*], M. Carriquiry [†], M. J. Gibb[‡], S. Tamminga[§], and P. Chilibroste[*]

4.1.1 Abstract

The objective of this study was to evaluate the effect of timing of corn silage supplementation on rumen pool size, rumen fermentation and *in situ* DM degradability on grazing dairy cows. Six rumen-cannulated Holstein dairy cows in a randomized block design grazed on a second year mixed grass-legumes pasture from 0900 to 1500 h and received 2.7 kg DM of a commercial concentrate at each milking. Herbage allowance was set at 15 kg DM/cow/day determined at ground level. The treatments involved the supplementation of 3.8 kg DM/day of corn silage offered in a single meal at 0800 h (**AM**), equally distributed in two meals at 0800 h and 1700 h (**AM-PM**) or in a single meal at 1700 h (**PM**). The experiment was carried out during late autumn and early winter, with three weeks of adaptation and five weeks of measurements. The total rumen pool tended to be greater in PM than in AM-PM and intermediate in AM cows, while the liquid:solid ratio was greater in AM and PM than in AM-PM cows. Organic matter pool sizes were greater in PM than AM and intermediate in AM-PM cows with a significant treatment by time-of-sampling interaction. Total volatile fatty acid, acetate and propionate pool sizes tended to be greater in AM than in AM-PM and intermediate in PM cows. Timing of corn-silage ration offer had a significant effect on mean rumen pH; being the highest in AM-PM, the lowest in PM and intermediate in AM cows (6.5, 6.1 and 6.4; respectively). The differences in rumen pH between treatments were significant during the non-grazing period from 1900 to 0900 h, with pH being below 6.0 for PM cows during nighttime samplings. Rumen ammonia concentration was greater in PM cows than in the other two treatments, showing a different pattern along the day with a significant interaction treatment by time-of-sampling. Offering corn silage before grazing (AM) would work as a ruminal fermentation buffer, but at the same time, it may operate as a satiety signal, which could reduce herbage DM intake rate during the grazing session. We concluded that timing of corn silage allocation impact rumen fill and fermentation due to changes in ingestive behaviour and total dry matter intake.

Keywords: feeding strategy, grazing, rumen fermentation, rumen pools, silage allocation

4.1.2 Implications

A more comprehensive knowledge about the effects of timing of corn silage supplementation in relation to grazing will support more precise and efficient feeding strategies. The results of this study constitute a valuable management tool and show that feeding dairy cows with whole crop corn silage in different moments related to the grazing session did impact rumen fill, fermentation kinetics and eventually milk production and composition. We have shown that it is possible to increase the output from a limited amount of feed inputs modifying the feeding strategy.

4.1.3 Introduction

Milk production is greatly determined by dry matter intake (**DMI**) and, to a lesser extent, by the efficiency of DM conversion into milk components (Chilibroste *et al*., 2005). In grazing dairy systems, exposed to large variations in grass growth between seasons, herbage allowance may become restrictive and limit DMI. In such circumstances cows may be offered silage and concentrate rations to satisfy their DMI requirements (Armstrong *et al*., 2010; Peyraud and Delagarde, 2013). In addition, restricting access time to pasture can be used as a grazing management tool to increase “grazing efficiency” (proportion of time cows spent grazing/actual access time) and pasture utilization (Chilibroste et al., 2015; Gregorini, 2012).

However, restricting herbage allowance or access time to pasture may reduce herbage DMI and milk production in dairy cows (Chilibroste *et al*., 2012; Mattiauda *et al*., 2013). Therefore, timing of supplement allocation with respect to the grazing session might impact herbage and total DMI and on rumen fermentation kinetics (Chilibroste *et al*., 2008). It has been postulated that a higher rumen fill may accelerate satiation (Chilibroste et al., 2015; Gregorini et al., 2009a; Kennedy et al., 2011), or conversely, a long fasting period before the grazing session could induce a greater motivation to graze (Chilibroste *et al*., 2007; Soca *et al*., 2014). These research antecedents integrated at a daily feeding strategy level could explain the observed changes in herbage intake rate and DMI due to timing of corn silage supplementation (Mattiauda *et al*., 2018).

Several studies have focused on the effect of timing of concentrate supplementation on DMI and performance in beef cattle (Adams, 1985; Gekara *et al*., 2005) and dairy cows (Mitani et al., 2012; Trevaskis et al., 2004), but only few have reported effects on rumen fermentation kinetics (Kolver *et al*., 1998; Trevaskis *et al*., 2004). Kolver *et al*. (1998) reported that synchronization of a diet based on mechanically harvested fresh pasture with non-structural carbohydrate from a corn-based supplement reduced mean ruminal pH and variation as well as ammonia concentration pattern, but did not affect volatile fatty acid (**VFA**) concentrations, nitrogen (**N**) efficiency, DMI or animal performance. Similar to the previous experiment, Trevaskis *et al*. (2004) showed that synchronizing N availability with that of rapidly fermentable carbohydrates in grazing dairy cows reduced rumen ammonia after pm milking without a significant impact on production responses.

In vitro studies have shown that average rumen pH was lower and propionate concentration was higher when corn silage was supplemented 9 h rather than 1 h before a short ‘simulated meal’ of herbage (Gregorini *et al*., 2010). These authors suggested that under the same herbage allocation, a simple change in timing of supplementation may improve utilization of nutrients supplied by the pasture. Similarly, Mitani *et al*. (2005) reported a greater milk N output when dairy cows were fed with corn-silage-based supplement before, rather than immediately after, the grazing session. When dairy cows were fed corn silage 2 or 9 h before the grazing session, herbage DMI differed, but did not affect either milk yield or milk composition (Al-Marashdeh *et al*., 2016a). Félix *et al*. (2017) reported that restricted access to pasture resulted in a more variable pH and ammonia ruminal pattern in beef heifer compare with unrestricted possibly due a more stable ingestion pattern throughout the day for the latter. Our hypothesis was that splitting the corn-silage supplement into two meals (before and after grazing) rather than offering it in a single meal either before or after the grazing session, would impact rumen fermentation kinetics and daily rumen fill pattern. Thus, the objective of this study was to evaluate the effect of timing of corn silage supplementation on rumen pool size, rumen fermentation and *in situ* DM degradability in grazing dairy cows.

4.1.4 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 three weeks of adaptation (week 0 to 2) and five weeks of measurements (week 3 to 7). Animal procedures were approved by the Animal Experimentation Committee of the University of Uruguay.

Six rumen-cannulated autumn-calving multiparous Holstein cows, 2.3 ± 0.49 (means ± SD) lactations were used. At the beginning of the study, cows were at 39 ± 18 days in milk, weighed 514 ± 42.5 kg and produced 19.7 ± 4.11 kg/day of milk. Cows were blocked by body weight, milk yield and days in milk and randomly assigned to one of three treatments in which a daily ration of 3.8 kg DM of whole-crop corn silage was offered in a single meal at 0800 h (**AM**) equally distributed in two meals offered at 0800 and 1700 h (**AM-PM**) or in a single meal at 1700 h (**PM**).

Cows grazed 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 of 1 540 ± 176.4 kg DM/ha (measured to ground level). All cows (separated by treatment) had access to a daily strip of ungrazed pasture from 0900 to 1500 h and received 2.7 kg DM of a commercial ground sorghum-grain-based concentrate at each milking. Daily strips of pasture for grazing were adjusted to provide a daily herbage allowance of 15 kg DM/cow based on the measurement of the pre-grazing herbage mass (DM kg/ha). 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 two consecutive days were weekly collected to determine milk fat, protein, and lactose concentration with a MilkoScan (Foss Electric®, Model 133b, Hillerød, Denmark).

Herbage mass and chemical composition

To determine the appropriate strip areas, herbage mass was calculated weekly using a double sampling technique as described by Mattiauda *et al.* (2018). Every week, the herbage mass was calculated by measuring the sward plate height with the rising plate meter (**RPM**) at 50 points within the paddocks. During weeks 3, 5, and 7, 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.

Rumen evacuations

Rumen evacuations were performed during week 4 and 6 following the scheme presented in Table 1. Two cows per time-of-sampling were moved to a small enclosed area on the side of the milking parlour and their rumen was evacuated simultaneously. Before emptying the rumen, samples of ruminal fluid were collected to measure ammonia and VFA concentrations. All rumen contents that could be removed by hand were emptied into a large container provided with a filter of 0.04 mm^2 covered by a double cheesecloth to separate solid from liquid fractions. After removal of rumen contents, fractions were weighted and the liquid to solid ratio was calculated to determine rumen pools. Sub-samples of the solid and liquid fractions were reconstituted according with this ratio to provide a representative sample of the rumen content. The rumen content sample was frozen at -20 °C until determination of DM, ash, and N contents. After the samples of the rumen content were collected, all the remaining rumen content was placed back into the rumen, and the time recorded.

Table 1 Rumen evacuation scheme (day and time-of-sampling) repeated during weeks 4 and 6 of the experiment for 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)

Time	Tuesday	Wednesday	Thursday	Wednesday
0500 h		AM	AM-PM	PM
1530 h		AM-PM	PM	AM
2000 h	PM	AM	AM-PM	

Rumen fermentation

Ruminal fluid samples were collected at 0900, 1030, 1200, 1400, 1730, 1900, 2100, 2300 h, and at 0700 h the following day during one 22-h period in weeks 3, 5 and 7 to determine pH, ammonia and VFA concentrations. For rumen sample collection, fistulated dairy cows were moved, depending on sampling time, to a dry-lot pen adjacent to the grazing plots or to the milking parlour to minimize disruption on cows' activity. Rumen samples were filtered through a cheesecloth and an aliquot sample was taken immediately to measure pH using a portable pH-meter (Oakton, Eutech Instruments, Malaysia). Two other aliquot samples were acidified in ratio of a 20:1, with sulphuric (95.6%) or orthophosforic (85%) acids and frozen for ammonia and VFA analyses, respectively. Ammonia was determined by distillation with MgO (Bremner, 1960) and VFA by gas-chromatography as described by Chilibroste *et al*. (2000). The non-glucogenic to glucogenic ratio was calculated as the ratio of lipogenic (acetic and butyric) to glucogenic (propionic, isobutyric, valeric and isovaleric) VFA.

In situ degradability

To evaluate the effect of the ruminal environment the *in situ* technique was used (Ørskov *et al*., 1980). During week 3 and 5, porous polyester bags (size 12×7 cm; mean pore size 50µm) containing 4.5 g DM of lucerne (*Medicago sativa)* hay, milled using a 2 mm screen, were used. Before rumen incubation, bags were immersed for 15 min in warm distilled water at 39 °C. Bags were then introduced simultaneously into the rumen, immediately after the beginning of grazing session (0900 h), and removed sequentially (2 bags each time) after 4, 12, 24, 48, and 120 h of incubation. After removal from rumen, the bags were soaked in iced water. After drainage the bags were washed 5 times using an automatic machine, with a soft program with no centrifugation for 90 s, and frozen at -20 °C. After thawing, bags were dried in a forced air oven at 60 °C for 48 h and weighed. Dry matter losses were computed as the difference in DM weight of the pre- and post-incubated bags, and expressed as the proportion of initial weight.

Chemical composition

All samples (feedstuff and rumen samples) for chemical analysis were ground through a 1 mm screen. Dry matter (AOAC7 967.03), ash (AOAC7

942.05), and nitrogen (AOAC7 984.13) contents were determined according to the procedure of the AOAC (1990); and *in vitro* digestibility as described by Tilley and Terry (1963). Organic matter (**OM**) was calculated as DM minus ash. Crude protein was calculated as N × 6.25. The neutral detergent fibre (aNDFom) was determined with heat stable amylase and expressed as ash free; acid detergent fibre (ADFom) also was expressed as ash free. All fibers were determined according to Van Soest *et al*. (1991).

Calculations and statistical analysis

Data was analyzed in a randomized block design using the SAS Systems program package (v. 9.2, SAS Institute Inc., Cary, NC, USA). Milk yield and composition were analyzed with 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 × week interaction as fixed effects and blocks as random effects. Rumen pools parameters were analysed with the MIXED procedure and a model that included treatment, time-of-sampling, and their interaction as fixed effects and week and block as random effects. Rumen fermentation parameters were analyzed using the MIXED procedure with a model that included week, treatment, time-of-sampling, and their interaction as fixed effects and block as a random effect. For analyzed rumen pools and fermentation variables, the spatial power (SP (POW)) was included as the covariance structure and the Kenward-Rogers procedure was used to adjust the denominator degrees of freedom.

To estimate degradation parameters, data of DM disappearance after different incubation times were fitted to the Ørskov and McDonald (1979) model as follows:

$$Y_{(t)} = a + b\,(1 - e^{-kdt}),\ t \geq 0$$

where: $Y_{(t)}$=fraction disappearance at time t, a=soluble or rapidly degradable fraction, b=insoluble but potentially degradable fraction, kd=degradation rate (h^{-1}), t=incubation time (h). Degradation parameters were calculated using a non-linear procedure with Marquardt method (NLIN procedure) and rumen degradation parameters were analysed using the MIXED procedure with a model including treatment as a fixed effect and block as a random effect. Least square means were separated using Tukey-Kramer tests (alpha = 0.05) and means were considered to differ if $P \leq 0.05$ and tendencies were declared if $0.05 < P \leq 0.10$.

4.1.5 Results

Milk yield and chemical composition of herbage and supplements

The yield of 4% fat corrected milk (**FCM**), milk fat, protein and lactose concentration were not different between treatments: 22.9 ± 3.15 kg/day, 37.5 ± 0.50 g/kg, 29.9 ± 0.17 g/kg and 47.9 ± 0.13 g/kg for FCM, fat, protein and lactose, respectively. Results of the chemical analyses of the herbage sampled as representative of that eaten by the cows, and of the corn silage and concentrate supplements are shown in Table 2.

Rumen evacuation

Results of rumen liquid, solid, total, OM and nitrogen contents are shown in Table 3. Treatment had a significant effect ($P < 0.05$) on the weight of liquid within the rumen been greater in AM than AM-PM cows and intermediate for PM cows (Table 3) with a significant treatment × sampling time interaction. Overall, the weight of the solid fraction was slightly greater ($P < 0.08$) in PM than AM cows and intermediate for the AM-PM cows. However, there was an effect of sampling time, with the weight of solids being significantly lower in the samples collected at 0500 h than those collected at 1530 h and 2000 h. There was a treatment × sampling time interaction effect of the weight of the solid fraction. Treatment had a significant effect ($P < 0.05$) on rumen DM content being 10.1, 10.9, and 11.0

± 0.56 kg for AM < AM-PM and PM, respectively. The weight of OM in the rumen was affected by treatment, sampling time and a treatment × sampling time interaction. Although the rumen nitrogen content was not affected by treatment, it did differ between sampling times (Table 3).

Table 2 Chemical composition[1] of herbage collected as representative of that selected by the cows, and of corn silage and concentrate offered to dairy cows

Item	Herbage	Corn Silage	Concentrate
DM, (g/kg)	183 ± 1.28	256 ± 0.10	901 ± 0.85
OM[1], (g/kg DM)	908 ± 0.79	930 ± 0.05	918 ± 0.21
CP, (g/kg DM)	225 ± 1.65	68.1 ± 0.16	173 ± 0.22
aNDFom[1] (g/kg DM)	307 ± 1.15	566 ± 0.40	248 ± 0.14
ADFom[1] (g/kg DM)	203 ± 1.15	317 ± 0.17	99.3 ± 0.05
OM digestibility *in vitro*, (g/kg DM)	775 ± 1.50	745 ± 2.12	782 ± 1.20

[1] OM, organic matter; aNDFom, neutral detergent fibre assayed with a heat stable amylase expressed exclusive of residual ash; ADFom, acid detergent fibre expressed exclusive of residual ash.

Values of ammonia, total VFA, acetate, propionate and butyrate contents of the rumen liquid-phase pool are shown in Table 4. The rumen ammonia pool was affected by both treatment and sampling time, and showed a treatment × sampling time interaction. The ammonia pool was consistently lower in cows on treatment AM-PM than those on the other two treatments, and was consistently higher when sampled at 1530 h compared with 0500 h and 2000 h. There was a tendency ($P \leq 0.08$) for total VFA, acetate and propionate pools to show an overall effect of treatment, being greater AM than AM-PM and intermediate in PM cows. Total VFA, acetate, propionate and butyrate pools were not affected by the time of sampling. The butyrate pool showed no effect of treatment.

Table 3 Rumen contents and pools in grazing 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), effect of treatment (T) and sampling time (St)

Item	St	Treatment (T)			St	SEM	*P*-value		
	(hour)	AM	AM-PM	PM	Mean		T	St	T×St
Content (kg)									
Liquid	0500 h	19.7^{Y}	17.9	25.3	21.0				
	1530 h	28.0^{aX}	18.1^{b}	22.6^{ab}	22.9				
	2000 h	25.5^{X}	23.2	21.9	23.4				
	T mean	24.4^{a}	19.7^{b}	23.2^{ab}	-----	2.74	0.02	0.30	0.03
Solid	0500 h	35.8	40.0	40.6	38.8^{Y}				
	1530 h	66.7	68.1	59.0	64.6^{X}				
	2000 h	60.0^{b}	63.6^{b}	76.6^{a}	66.7^{X}				
	T mean	54.1^{y}	57.2^{xy}	58.7^{x}	-----	3.42	0.08	<0.01	<0.01
Total	0500 h	55.5	57.9	65.9	59.7^{Y}				
	1530 h	94.6^{a}	86.2^{ab}	81.6^{b}	87.5^{X}				
	2000 h	85.5^{b}	86.6^{b}	98.2^{a}	90.1^{X}				
	T mean	78.5^{xy}	76.9^{y}	81.9^{x}	-----	2.69	0.08	<0.01	<0.01
OM[1]	0500 h	4.4	5.2	5.4	5.0^{Z}				
	1530 h	8.6	8.6	7.5	8.2^{Y}				
	2000 h	8.3^{b}	8.9^{b}	11.5^{a}	9.6^{X}				
	T mean	7.1^{b}	7.6^{ab}	8.2^{a}	-----	0.35	<0.01	<0.01	<0.01
Nitrogen	0500 h	0.12	0.14	0.15	0.14^{Z}				
	1530 h	0.35	0.34	0.30	0.33^{Y}				
	2000 h	0.34^{ab}	0.33^{b}	0.41^{a}	0.36^{X}				
	T mean	0.27	0.27	0.29	-----	0.022	0.26	<0.01	<0.01

[1] OM= organic matter

[a,b] Means within a row with different superscripts differ ($P < 0.05$)

[x,y] Means within a row with different superscripts differ ($P < 0.10$)

[A,B] Means within a column with different superscripts differ ($P < 0.05$)

[X,Y,Z] Means within a column with different superscripts differ ($P < 0.10$)

Rumen fermentation

Results of the analysis of rumen fluid samples collected on nine occasions over 22 h in weeks 3, 5 and 7 are shown in Table 5. There were no differences between weeks in any of the parameters measured so the mean values presented are those pooled over the three 22-h periods. Both pH and ammonia concentration were affected by treatment, time of sampling, and a treatment × time-of-sampling interaction, as illustrated in Figure 1.

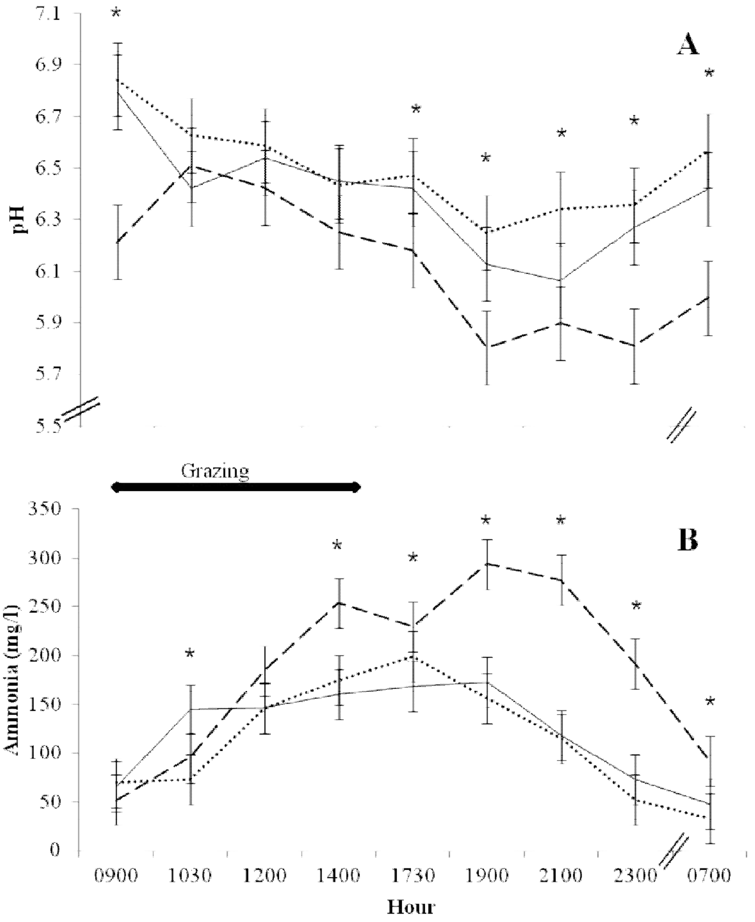

Figure 1 Diurnal patterns of ruminal pH (A), and ammonia (B) concentrations (mg/l) of strip-grazing dairy cows offered corn silage at 0800 h (AM, ——), equally offered at 0800 and 1700 h (AM-PM, ······) or at 1700 h (PM, - - - -). Within a sampling time, * indicate at least one difference among treatments ($P < 0.05$).

Table 4 Rumen liquid-phase pool of ammonia and volatile fatty acid (VFA) in grazing 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), effect of treatment (T) and sampling time (St)

Item	St	Treatment (T)			St	SEM	*P*-value		
	(hour)	AM	AM-PM	PM	Mean		T	St	T×St
Liquid-phase pool									
Ammonia, g	0500 h	2.35	1.39	1.62	1.79^{BZ}				
	1530 h	6.35^{a}	3.54^{c}	4.99^{b}	4.96^{AX}				
	2000 h	2.33^{ab}	1.82^{b}	3.71^{a}	2.62^{BY}				
	T mean	3.68^{a}	2.25^{b}	3.44^{a}	-----	0.676	<0.01	<0.01	0.03
VFA pool, Mol									
Total VFA	0500 h	3.42	2.45	4.23	3.37				
	1530 h	3.76	2.80	3.16	3.24				
	2000 h	4.14	2.76	2.87	3.26				
	T mean	3.78^{x}	2.67^{y}	3.42^{xy}	-----	0.625	0.08	0.96	0.46
Acetate	0500 h	2.14	1.58	2.80	2.18				
	1530 h	2.47	1.80	2.06	2.11				
	2000 h	2.67	1.77	1.90	2.11				
	T mean	2.43^{x}	1.72^{y}	2.25^{xy}	-----	0.308	0.08	0.97	0.38
Propionate	0500 h	0.73	0.48	0.84	0.67				
	1530 h	0.74	0.57	0.62	0.64				
	2000 h	0.87	0.57	0.54	0.66				
	T mean	0.78^{x}	0.54^{y}	0.67^{xy}	-----	0.136	0.09	0.93	0.46
Butyrate	0500 h	0.44	0.30	0.47	0.40				
	1530 h	0.43	0.32	0.37	0.38				
	2000 h	0.46	0.33	0.31	0.36				
	T mean	0.45	0.32	0.38	-----	0.084	0.15	0.81	0.72

^{a,b} Means within a row with different superscripts differ ($P < 0.05$)

^{x,y} Means within a row with different superscripts differ ($P < 0.10$)

^{A,B} Means within a column with different superscripts differ ($P < 0.05$)

^{X,Y,Z} Means within a column with different superscripts differ ($P < 0.10$)

Treatment showed a tendency ($P \leq 0.08$) to affect acetate, propionate, and total VFA concentrations, with samples from treatment PM consistently having higher concentration than those from AM and AM-PM. As a result, the concentrations of lipogenic and glucogenic precursors showed a tendency to be higher in the PM cows ($P \leq 0.09$). Treatment did not affect the lipogenic/glucogenic precursor ratio (Table 5).

Table 5 Rumen fluid pH, and ammonia and volatile fatty acid (VFA) concentrations in grazing 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), effect of treatment (T) and sampling time (St)

Item	Treatments			SEM	*P*-value		
	AM	AM-PM	PM		T	St	TxSt
pH	6.4^{b}	6.5^{a}	6.1^{c}	0.12	<0.01	<0.01	0.05
Ammonia (mg/l)	122.0^{b}	113.1^{b}	185.6^{a}	13.04	<0.01	<0.01	0.01
VFA concentration (mmol/l)							
Acetate	101.7^{y}	101.6^{y}	110.3^{x}	3.10	0.08	0.43	0.47
Propionate	32.6^{xy}	31.3^{y}	34.9^{x}	1.25	0.07	0.21	0.33
Butyrate	19.0	19.5	21.4	0.89	0.13	0.21	0.28
Isobutyrate	1.33	1.31	1.34	0.059	0.94	0.09	0.48
Isovalerate	1.79	1.91	1.85	0.107	0.72	0.14	0.50
Valerate	1.97^{b}	2.04^{ab}	2.33^{a}	0.102	0.03	0.15	0.41
Total	158.3^{xy}	157.6^{y}	172.1^{x}	3.84	0.08	0.33	0.39
Lipogenic	120.6^{y}	121.1^{y}	131.7^{x}	1.35	0.08	0.40	0.42
Glucogenic	37.7^{xy}	36.6^{y}	40.5^{x}	0.23	0.09	0.18	0.32
Lipogenic/glucogenic	3.25	3.36	3.27	5.053	0.17	0.54	0.34

[a,b] Means within a row with different superscripts differ ($P < 0.05$)

[x,y] Means within a row with different superscripts differ ($P < 0.10$)

In-situ degradability

Treatment had no effect neither on the potential degradability of the DM in the lucerne hay (proportionately 0.55, 0.56 and 0.56 ± 0.010 for AM, AM-PM and PM, respectively), nor on the degradation rate (0.078, 0.091, and 0.079 ± 0.006 h^{-1} for AM, AM-PM and PM, respectively) (Figure 2). Thus the estimated effective degradability of DM (kp = 0.08 h^{-1}) did not differ between treatments (proportionately 0.49, 0.46, and 0.48 ± 0.023 for AM, AM-PM and PM, respectively).

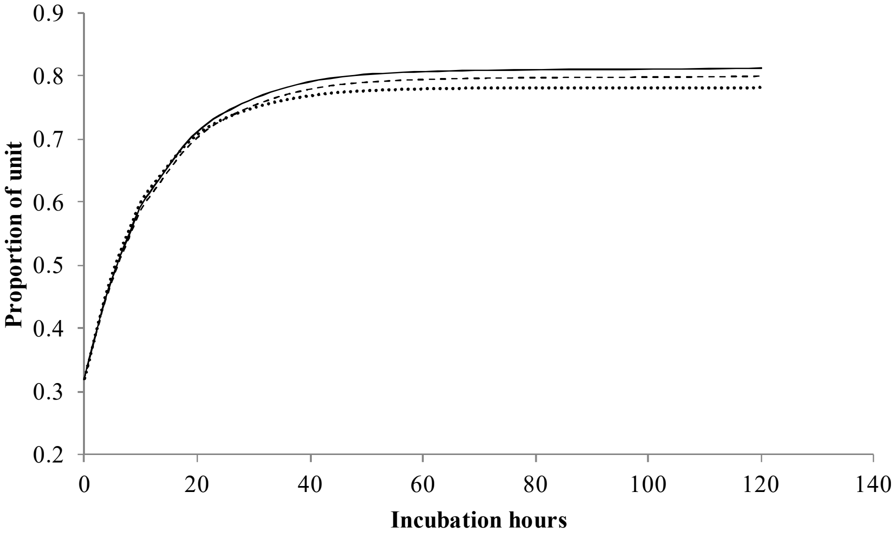

Figure 2 Dry matter degradability *"in situ"* of lucerne hay of strip-grazing dairy cows offered corn silage at 0800 h (AM, ——), equally offered at 0800 and 17:00 h (AM-PM, ······) or at 1700 h (PM, - - - -).

4.1.6 Discussion

Timing of corn silage supplementation affected rumen kinetic as demonstrated by the mean values and temporal patterns of change in pH, ammonia concentration and the rumen liquid to solid fraction ratios. The weight of the liquid fraction slightly differed (P < 0.10) between the three times of sampling only in AM cows, and between treatments only at 1530 h when the cows were removed from the pasture (AM > PM ≥ AM-PM). The increase in the liquid fraction and thus a lower DM rumen content has been associated with a lower DM rumen pool size and a lower buffer capacity of the rumen (Chilibroste *et al*., 2001). In contrast, the weight of the solid and OM fractions differed between the different sampling times, depending upon the treatment imposed. After returning from pasture, the weight of the solid and OM fractions had increased appreciably and, although somewhat lower in the PM cows, did not differ significantly due to treatment. However, by the evening sampling (2000 h), following consumption of their entire corn silage ration, the solid and OM fractions in PM cows had increased above that of cows on treatments AM and AM-PM. These increases could respond to the corn silage consumed after return from pasture added to the higher herbage DMI reported by Mattiauda *et al*. (2018) in a related productive experiment. The effect of the ingestion pattern (herbage + supplements) on rumen fill dynamics is in line with previous studies (Chilibroste et al., 2001; Gregorini et al., 2010, 2009b).

The greater ammonia pool size observed in PM cows compared with AM-PM cows may be due to the sequence of feeds (herbage + corn silage *vs* half corn silage + herbage + half corn silage, for PM and AM-PM treatments, respectively) and their mixing and kinetics at rumen level. In line with this hypothesis, PM cows exhibited a higher rumen ammonia concentration with the larger differences being observed after the grazing session. AM-PM cows probably experienced a better synchrony of nutrients (Kolver *et al*., 1998) and improved efficiency at rumen level (Trevaskis *et al*., 2004), which is consistent with the tendency for higher FCM and higher body condition score in AM-PM cows (Mattiauda *et al*., 2018). In the present experiment milk yield and FCM and milk composition were not different between treatments, probably due to the low number of cows involved in this experiment. On the other hand, ammonia pool size observed in AM cows was not different than PM cows, while rumen ammonia concentration of AM cows was similar to

AM-PM probably due to a lower synchrony along the day of the once a day (AM and PM) versus twice a day corn silage supplementation (AM-PM).

The greater total VFA rumen pool size for AM than for AM-PM could reflect a different balance between production and absorption of VFA. Besides, the reduced VFA pool size for AM-PM cows could also be related to the smaller size of the liquid fraction in this treatment as reflection of a more stable rumen conditions throughout the day (Chilibroste *et al*., 2001).

The overall pattern of change in solid fraction and OM pool sizes throughout the day is a reflection of the cumulative patterns of ingestion of the different dietary components (Chilibroste *et al*., 2001), whereas the ammonia pool size pattern which peaked for all the treatments at the end of the grazing session (1530 h) could be a reflection of the cumulative herbage intake. However, the treatment by sampling time interaction showed that the ammonia pool was probably related to the nitrogen ingestion since AM cows reached extreme values at 1530 h, PM cows did at 2000 h and AM-PM cows showed lower and less extreme values at all sampling times. For the AM-PM cows there was only a moderate increase in the rumen ammonia pool sampled at 1530 h, followed by a decline when measured at 2000 h. This relatively small fluctuation in ammonia pool size, and the ammonia concentrations and pH values measured in the nine samples aspirated over 24 h, are evidence of a more stable rumen environment compared with those pertaining to the other two treatments (Chilibroste *et al*., 2008; Félix *et al*., 2017). In contrast, cows on AM treatment having received their entire silage ration before grazing and consumed less herbage than the AM-PM cows (Mattiauda *et al*., 2018), had a much greater rumen pool when sampled at 1530 h which declined rapidly by 2000 h. Such large fluctuations in the ammonia pool can suggest a rapid release of N from the ingested silage and herbage, and probably a different rate of incorporation into microbial protein. Having received no silage before grazing and consuming only slightly less fresh herbage than AM-PM cows, the PM cows had accumulated a rumen ammonia pool intermediate between the AM-PM and AM cows by the end of grazing. However, having received their full silage ration at 1700 h, the PM cows' rumen ammonia pool was larger than that of cows on the other two treatments at 2000 h, and their rumen ammonia concentration, although declining during the night, remained higher than in the AM and AM-PM cows. These results that can be a consequence of the different ingestive pattern and synchronization of nutrients along the day differed from Mitani *et al*. (2005) who reported no differences in ammonia concentrations in rumen when a corn-silage based supplement was offered to cows before or after the grazing session. This lack of agreement between experiments could be due to the fact that in this study, grazing was split in two restricted grazing sessions (2.5 h each), and pasture represented 42% of the whole diet, while in Mitani *et al*. (2005), pasture represented 52% of the diet. However, *in vitro* rumen ammonia concentration was reduced when corn silage was included before pasture (Gregorini *et al*., 2010) in a experiment with similar diet and proportion than in our study.

Overall, rumen pH values were higher when corn-silage ration was offered in two meals before and after grazing (AM-PM cows), than when it was offered in one meal, either before (AM) or after (PM) grazing. Although statistically significant, the differences in rumen pH between AM-PM and AM cows were relatively small and showed a similar pattern over 24 h. In contrast, following release to pasture, rumen pH in the PM cows rose from the initially low level to levels similar to those of the AM and AM-PM cows, but by the end of their time at pasture, rumen pH in PM cows started to decline below those of cows on the other two treatments. Although rumen pH recovered slightly during the evening, it remained lower than in the AM and AM-PM cows until the following morning. This indicates that compared with cows on PM treatment, rumen conditions in cows on AM and AM-PM treatments, having consumed all or part of their silage ration prior to being released to pasture, might exhibit a higher buffer capacity for the effect of herbage ingestion, both during the grazing session and subsequently (Chilibroste *et al*., 2001). Thus the higher DMI (herbage + corn silage) and the ingestion pattern throughout the day of PM cows (Mattiauda *et al*., 2018), could explain the lower pH nadir and the longer time at low pH values observed in these cows (Mattiauda *et al*., 2013). Although PM cows maintained ruminal pH below 6.2 for almost 12 h, no differences were detected between treatments in the *in sacco* degradability of lucerne, the milk fat content or the fat yield determined in these cows which is in line with the productive experiment reported by Mattiauda *et al*. (2018). The pH pattern of AM-PM cows suggest that timing of corn silage supplementation could have modulate fermentation kinetics with a more stable rumen pH throughout the day and ultimately with a higher herbage DMI (Mattiauda *et al*., 2018). As expected, rumen ammonia concentration was inverse to pH, with PM cows exhibiting greater concentrations when compared with AM-PM and AM cows, probably due to observed changes on DM and N intake (Mattiauda *et al*., 2018). Indeed, PM cows showed a higher increase in ammonia concentrations at the beginning of the grazing session (1030 until 1730 h) when compared with the other treatments, which was probably due to the asynchrony of the diet ingredients as when cows began to graze, their rumen was emptier (Al-Marashdeh et al., 2016b; Gregorini et al., 2010).

However, in agreement with Mitani *et al*. (2005), total VFA concentrations tended to be greater in PM cows when compared to the other two treatments. This could be the result of the high herbage and total DMI as well as the different ingestive pattern experienced by this cow (Mattiauda *et al*., 2018). In contrast, when corn silage was offered before the grazing session (AM cows), while it appeared to buffer ruminal fermentation at the same time, it may trigger satiety signal (Gregorini, 2012), impacting negatively on herbage DM intake (Gregorini *et al*., 2009b). Whilst acetate concentrations tended to be greater for PM cows compared with the other treatments, there was no evidence of any treatment effect on the lipogenic to glucogenic VFA ratio, as expected from differences in the FCM yield and composition reported by Mattiauda *et al*. (2018).

4.1.7 Conclusions

Timing of corn silage allocation in respect to the grazing session did impact on rumen fill and fermentation kinetics. Splitting the corn silage into two meals (before and after the grazing session) maintains more stable rumen conditions based on pH, ammonia and VFA rumen pools and concentration. The apparent more stable rumen conditions did not affect the degradation pattern of alfalfa hay, but might have been one of the main factors to support the higher DMI reported by Mattiauda *et al*. (2018) in a related productive experiment.

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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 is not deposited in an official repository.

4.1.8 References

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5. DISCUSIÓN GENERAL

La producción y utilización de forraje tiene un alto impacto en la competitividad de los sistemas de producción de leche de Uruguay (Chilibroste, 2015). La baja producción de forraje en el período otoño/invierno es limitante para satisfacer la demanda de alimentos debido a la reducción del área efectiva de pastoreo y a la concentración de partos que se da en ese período. Adicionalmente, los sistemas lecheros han tendido a aumentar la carga animal en los últimos años (DIEA, 2017). La pastura cosechada en forma directa tiene baja participación en las dietas en este período del año lo que determina el uso de suplementos de manera estructural en la mayoría de los sistemas lecheros, y destaca la importancia de hacer un uso eficiente de los mismos. En este sentido, es fundamental estudiar estrategias de manejo que mejoren la eficiencia del uso de estos recursos (pastura y suplementos); ya sea a través del manejo del pastoreo y/o de la suplementación para mejorar tanto el CMS como la eficiencia del uso de los nutrientes y consecuentemente los resultados productivos.

5.1 ESTRATEGIA DE ALIMENTACIÓN Y CONSUMO

Cuando se estudió el tiempo de acceso al pastoreo (Capítulo 2), el CF de las vacas que accedieron al pastoreo por 4 h (T7-11 y T11-15) fue aproximadamente 20% menor que aquellas que lo hicieron durante 8 h (T7-15). Estos resultados indican que 4 h de pastoreo no fueron suficientes para que las vacas compensaran el CF a través de alguno de los mecanismos que permiten aumentar la tasa de consumo. Si bien la asignación utilizada en este trabajo (20,7 ± 2,46 kg MS/v/d) no parece haber sido la principal limitante al CF (Baudracco et al., 2010, Peyraud et al., 1996), la baja capacidad de compensación observada puede ser explicada por limitantes en la masa de forraje (1590 ± 161 kgMS/ha) y/o en características de estructura y densidad (Soca et al., 1999). El efecto de restricciones severas en el tiempo de acceso a la pastura en el CF ha sido poco estudiado. Pérez-

Ramírez et al. (2008) y Kristensen et al. (2007) reportaron una reducción similar (18,6% y 18,1%; respectivamente) cuando el acceso al pastoreo se redujo de 8 a 4 h o de 9 a 4 h respectivamente. Si bien los rumiantes presentan una gran adaptación a la restricción en el tiempo de acceso al pastoreo (Chilibroste et al., 2015), en estas condiciones el tiempo de acceso no fue suficiente para igualar el CF con los animales expuestos a una restricción más moderada.

Cuando se estudiaron diferentes momentos de suministro de ensilaje de maíz respecto al pastoreo restringido de 6 h (Capítulo 3), las vacas que lo recibieron previo al pastoreo consumieron 26% menos forraje que aquellas a las que se les suministró la misma cantidad de ensilaje antes y después del pastoreo (8,5 vs. 11 kgMS; respectivamente), mientras que el CF de las vacas que recibieron el ensilaje de maíz después del pastoreo fue intermedio (10,3 kgMS). De forma similar, Al-Marashdeh et al. (2016a) reportaron que el CF fue menor cuando las vacas recibieron el ensilaje de maíz 2 h respecto a 9 h antes del ingreso al pastoreo. En contraste con nuestros resultados, Mitani et al. (2005) no encontraron diferencias en el consumo de forraje en respuesta al momento de la suplementación cuando las vacas accedieron al pastoreo por 5 h totales repartidas en dos turnos. Del mismo modo en vacas con 24 h de acceso a la pastura ya sea con (Sheahan et al., 2013) o sin (Trevaskis et al., 2004) acceso a una parcela nueva no encontraron efectos del momento de la suplementación en el CF. Probablemente en estos últimos experimentos, ya sea por el número de turnos de pastoreo (Santana et al., 2017) y/o por el tiempo de permanencia en la pastura, las vacas compensaron el CF atenuando el efecto del momento de suplementación (Gibb et al., 2000).

En las condiciones de nuestros experimentos, la restricción de 8 a 4 h (Capítulo 2) representó una disminución en el CF de 0,42 kgMS/h por cada hora de restricción de acceso al pastoreo. Sin embargo, el suministro de ensilaje de maíz antes del pastoreo resultó en una tasa de sustitución (TS) de 0,64 kg/kg, mientras que cuando se ofreció la misma cantidad en la tarde la TS fue de 0,18 kg/kg, tomando como testigo el tratamiento en que se repartió el ensilaje de maíz antes y después del pastoreo donde las vacas lograron el mayor CF (Capítulo 3). Estos resultados confirman la relevancia de la secuencia y momento en que se ofrecen los alimentos para mejorar la eficiencia de uso de un mismo recurso, y evidencia que los efectos y/o respuestas no son lineales o aislados sino que resultan de interacciones entre diferentes factores como se analizará más adelante.

5.2 CONSUMO, PRODUCCIÓN Y COMPOSICIÓN DE LA LECHE

El CMS logrado por las vacas está directamente relacionado a los resultados productivos que éstas pueden alcanzar en forma individual, y en nuestro trabajo el consumo de forraje fue el que determinó las diferencias en CMS total. Por esta razón, las vacas que accedieron al pastoreo por 4 h produjeron 1,3 kg/d (5,1%) menos de leche que aquellas con acceso durante 8 h. Pérez-Ramírez et al. (2008), reportaron reducciones similares en cuanto a producción de leche (1,1 kg/d; 5%) cuando el acceso al pastoreo se redujo de 8 a 4 h, mientras Kristensen et al. (2007) en vacas con mayor nivel de producción encontraron una disminución de 32,4 a 30,3 kg/d (6,5%) al reducir el acceso al pastoreo de 9 a 4 h. Por otro lado, Kennedy et al. (2009) probaron diferentes tiempos de accesos (6 a 22 h) y si bien encontraron diferencias en CMS a favor de las 22 h, esto no se reflejó en producción y composición de leche, destacándose que en este caso las vacas estaban en lactancia avanzada y un consumo potencial de forraje (13-14 kg) alcanzable con las restricciones impuestas y la suplementación utilizada.

Sin embargo y a pesar de lo esperado en nuestra hipótesis, no se encontraron diferencias en producción de leche en función del momento en que se ubicaron las 4 h de pastoreo, temprano (T7-11) vs tardío (T11-15).

No obstante, las vacas que accedieron más tarde al pastoreo (T11-15) produjeron 5,7% más proteína comparado a las que pastorearon temprano (T7-11). De forma similar, cuando se ofreció parcelas nuevas am y pm, Abrahamse et al. (2009) reportaron similar producción de leche pero mayor concentración y producción de grasa en vacas que ingresaban a la nueva parcela pm, asociado a una composición diferencial (am vs pm) de carbohidratos solubles de la planta. Sin embargo, en experimentos con dos turnos de pastoreo (total 6 h), Soca et al. (1999) encontraron una tendencia a aumentar la producción de leche (**PL)** y a bajar el contenido de grasa cuando la mayor sesión de pastoreo se ubicó en la tarde asociado a una mayor tasa de consumo.

La suplementación es otro aspecto que interactúa con el manejo del pastoreo en la eficiencia de uso de los recursos, y si bien ha sido ampliamente estudiada en vacas lecheras en pastoreo en lo que tiene que ver con niveles y tipo de suplemento, existen pocas evidencias del efecto del momento del día en que se realiza la suplementación en los resultados productivos, ya sea con concentrados (Sheahan et al., 2013) o ensilaje de planta entera de maíz (Al-Marashdeh et al., 2016a). En nuestro trabajo los resultados encontrados con acceso restringido al pastoreo (6 h) mostraron una mejora productiva de las vacas en cuanto a concentración y producción de leche corregida por grasa (**LCG**) y condición corporal (**CC**), al ofrecer el ensilaje de maíz repartido en dos veces respecto a darlo en una sola toma antes o después del pastoreo. Estos resultados se explican principalmente por el mayor CF logrado para las vacas AM-PM del orden de 26% mayor respecto a AM, mientras que el aumento en LCG fue de 6,5% superior. Es interesante notar que posiblemente parte de ese mayor CMS se haya destinado al mantenimiento o recuperación de la CC dado que fue mayor en el grupo de vacas AM-PM respecto a las AM e intermedio en las vacas PM. Mitani et al. (2005) no encontraron diferencias ni en CMS ni en PL y encontraron un aumento del contenido de grasa en la leche de vacas alimentadas con un suplemento en base a ensilaje de maíz (en una relación 70:30 ensilaje:concentrado), cuando éste se ofreció después del pastoreo comparado con las vacas que lo recibieron antes, mientras que estas últimas produjeron más proteína y tuvieron una mayor retención de Nitrógeno. Es importante considerar las condiciones del trabajo de Mitani et al (2005), ya que si bien el tiempo de acceso al pastoreo fue similar al nuestro (5 h), el mismo se dividió en dos turnos iguales así como también el suplemento ofrecido en forma de dieta mezclada, antes o después del pastoreo. Estas apreciaciones son relevantes ya que los nutrientes no operan de forma aislada, existen efectos e interacciones diferentes debido a la sincronía o no de los nutrientes con cada ingesta.

En nuestro estudio no se encontraron diferencias en producción de leche, ni en sólidos o CC cuando el ensilaje se ofreció en una sola toma ya sea antes (AM) o después del pastoreo (PM) (Capítulo 3). Estos resultados coinciden con Al-Marashdeh et al. (2016a) con 5 h de acceso a la pastura quienes no encontraron diferencias en PL, ni en sólidos ni en CC cuando ofrecieron ensilaje de planta entera de maíz, 9 o 2 h antes del pastoreo. Estos autores reportaron además mayor CF (0,8 kgMS/d, 7%) para las vacas que recibieron el ensilaje 9 h antes del pastoreo, probablemente debido a la menor capacidad de respuesta de las vacas dado la avanzada etapa de lactancia (>28 semanas). En nuestro estudio las vacas que comieron ensilaje PM (14 h antes del pastoreo) presentaron un CF de 10,3 kgMS/d, que no alcanzó a ser significativamente mayor que el de las vacas AM (8,5 kgMS/d); es posible que el número de animales incluidos para la determinación de consumo fue una limitante.

5.3 MECANISMOS INVOLUCRADOS EN EL CONTROL DEL CONSUMO EN PASTOREO

Los rumiantes y en particular las vacas lecheras, muestran un patrón de pastoreo con una frecuencia diaria de entre 3 a 5 eventos siendo las comidas más importantes temprano y tarde en la mañana y a la última hora de la tarde (Gibb et al., 1999). Este patrón es flexible y está muy influenciado por las condiciones en que se manejan los animales por lo que responden con mecanismos de adaptación en la conducta integrando diferentes señales al sistema nervioso central (Gregorini, 2012, Gibb, 2006).

En nuestro trabajo, la capacidad de las vacas de expresar su patrón natural de actividad de pastoreo diario fue limitada, por la restricción del tiempo de acceso al pastoreo (de 8 a 4 hs, Capítulo 2 y de 6 h, Capítulo 3), que limitó los eventos más comunes e importantes que ocurren hacia la última hora de la tarde. En este sentido, tanto el manejo del pastoreo como el momento de la suplementación con ensilaje de maíz provocaron cambios en el consumo de forraje a través de la conducta en pastoreo, ya que produciría un estado interno diferente (con estímulos de hambre o saciedad) en la primera y más importante sesión de pastoreo (en nuestro caso única), lo que podría afectar la reacción percibida por las vacas ante una misma fuente de alimento (Gregorini et al., 2009a) y su respuesta en consumo de forraje. El menor CF encontrado en el tratamiento de 4 h respecto 8 h (Capítulo 2), es consistente con el menor tiempo de acceso al pastoreo como ha sido reportado previamente por Pérez-Ramírez et al. (2008). En este trabajo se reportó una disminución de 326 a 208 minutos en el tiempo de pastoreo para las vacas con acceso de 8 y 4 h respectivamente, presentando este último grupo un comportamiento muy similar al nuestro (211 ± 9,3 minutos).

Por otro lado, las vacas que pastorearon más tarde (T11-15) presentaron una reducción (36 minutos, 15%) en el tiempo de pastoreo que las vacas que pastorearon más temprano (T7-11), asociado con menos movimientos mandibulares de prehensión y totales. Sin embargo, no se encontraron diferencias en el consumo de pastura lo que sugiere que las vacas de T11-15 tuvieron posibilidad de hacer bocados de mayor tamaño con mayores tasa de consumo en tiempos cortos (2,1 vs 1,7 kg/h para T11-15 vs T7-11, respectivamente). Esta evidencia es consistente con estudios realizados previamente que demostraron que bajo condiciones relativamente constantes en las características de la pastura, las vacas aumentaron la masa de bocado y la tasa de consumo en la medida que progresaba el día (Gibb et al., 1998, Orr et al., 1997). Adicionalmente, este aumento en la tasa de consumo se puede asociar con los cambios en la composición química de la pastura ya que el contenido de MS y de los carbohidratos solubles aumentan hacia la tarde lo que resulta en mayores pesos de los bocados además de mayores TB tarde en el día (Orr et al., 2001, Delagarde et al., 2000). Esto ha sido interpretado como una estrategia óptima de pastoreo para cosechar la pastura de mayor digestibilidad, con altas concentraciones en carbohidratos solubles y MS (Taweel et al., 2004, Gibb et al., 1998). La tasa de consumo tendió a ser mayor en las vacas T11-15 por lo que además de dedicar menos tiempo es probable que usen menos energía para alcanzar el mismo CF que el T7-11, lo que puede explicar el mayor rendimiento de proteína y lactosa en la leche en el tratamiento T11-15.

Si bien Greenwood y Demment (1988) encontraron que las vacas ayunadas pastoreaban más tiempo que aquellas no ayunadas con un efecto marcado en el largo de la primer sesión de pastoreo, en nuestro trabajo no se encontraron diferencias en el largo de la primer sesión de pastoreo entre tratamientos, a pesar que los diferentes tiempos de ayuno pueden explicar parte de las respuestas observadas en la tasa de consumo. Reducir el tiempo de acceso al pastoreo de 8 a 4 h ya sea en vacas suplementadas

(Pérez-Ramírez et al., 2008) o de 16 a 8 h en vacas no suplementadas (Chilibroste et al., 2007), aumentó el tiempo efectivo que estas dedicaron al pastoreo de 52 a 74% o 68 a 82%, respectivamente. Por lo tanto, se propone que los rumiantes adaptan su comportamiento en pastoreo previendo posibles eventos para cumplir con sus requerimientos bajo determinadas condiciones (Chilibroste et al., 2015, Provenza, 1995, Baile and McLaughlin, 1987). Esta mayor eficiencia se lograría a través del aumento en el peso de bocado y disminución de la masticación (Chilibroste et al., 2015), lo que explicaría que la mayor tasa de consumo del tratamiento T11-15 puede haber sido efecto del tiempo de ayuno previo al pastoreo (Gregorini et al., 2008, Chilibroste et al., 2007, Patterson et al., 1998).

Por otra parte, Gibb et al. (2000) reportaron diferentes sesiones de pastoreo dependiendo del momento en que se suministra el concentrado en el día. En nuestro trabajo, el mayor CMS encontrado para AM-PM se podría explicar por una tendencia a observar una mayor proporción de vacas en pastoreo para este tratamiento. Las determinaciones de altura a través del plato (RPM) a medida que las vacas pastoreaban, no mostraron diferencias entre tratamientos lo que sugiere que no hubo modificaciones en la estructura de la pastura o si las hubo fueron muy pequeñas. Sin embargo, se ha demostrado que las vacas aumentan el tamaño de bocado en la medida que presentan un menor llenado ruminal (Gregorini et al., 2007, Chilibroste et al., 2000). Por lo tanto, el aumento en CF por parte de las vacas AM-PM respecto a las AM puede estar dado por una combinación de pequeños aumentos en el tamaño de bocado junto a una mayor actividad total de pastoreo como resultado del menor llenado ruminal al inicio de la sesión de pastoreo. La cantidad de suplemento consumido antes de ingresar al pastoreo tiene un efecto indiscutible en la actividad de rumia para las 6 h siguientes; con un aumento estimado de 7 minutos/kgMS de ensilaje de maíz consumido en la mañana (Capítulo 3). Esto es consistente con reportes que comparan vacas suplementadas o no, donde las primeras presentan más sesiones y mayor tiempo dedicado a rumiar (Pérez-Prieto et al., 2011, Sheahan et al., 2011). Sheahan et al. (2013) observaron en vacas con 24 h de acceso a la pastura que el tiempo de rumia fue mayor para las que recibieron el concentrado en la tarde, dado por un aumento en la rumia hacia la noche. Estos datos son de relevancia e implicancia práctica ya que determinan el ambiente ruminal y la eficiencia del uso de nutrientes.

5.4 FERMENTACIÓN RUMINAL Y USO DE NUTRIENTES

En el capítulo 2, el pastoreo tarde en la mañana (T11-15) produjo una reducción del pH ruminal a las 4 h del ingreso a la parcela, asociado al aumento de AGV y la concentración de amonio, lo que fue consistente con las mayores tasa de consumo y tiempos de ayuno (Chilibroste et al., 1998). Sin embargo, el pH ruminal no se modificó durante las primeras 8 h de iniciada la sesión de pastoreo para el tratamiento T7-11, lo que puede deberse a una menor tasa de consumo que determina un ambiente ruminal distinto al grupo T11-15. Además, se deben tener en cuenta los cambios en la composición de la pastura a lo largo del día tales como mayor contenido de MS y carbohidratos solubles (Orr et al., 2001, Delagarde et al., 2000). La mayor tasa de consumo asociada al ayuno previo en las vacas T11-15 y los cambios esperados en la composición de la pastura son dos factores esenciales que pueden explicar un mejor aprovechamiento de nutrientes y consecuentemente mayor proteína en leche.

El momento de suplementación con ensilaje de maíz respecto del pastoreo afectó la cinética ruminal en términos de pH, concentración de amonio, AGV y fracciones líquido/sólido. El pH ruminal fue mayor y más estable cuando el ensilaje de maíz se ofreció en dos comidas que cuando se hizo en una sola comida ya sea antes o después del pastoreo. El pH ruminal en las vacas PM cayó de forma marcada hacia el final del día, asociado a la sesión de pastoreo y ensilaje. Esto indica que el ingreso al pastoreo con parte o todo el ensilaje ofrecido, predispone al rumen a tener una mayor capacidad de amortiguar el efecto de la ingestión del forraje. Las vacas PM mantuvieron un pH ruminal por debajo de 6,2 por más de 12 h, sin embargo, no se encontraron diferencias en la degradabilidad de la MS de alfalfa incubadas in sacco, contenido de grasa de la leche o producción de grasa para los diferentes tratamientos. La concentración de amonio en rumen se comportó en forma inversa al pH con mayores concentraciones para las vacas PM comparadas con las vacas AM-PM y AM, lo que refleja una menor sincronización de nutrientes de la dieta debido posiblemente a que los rúmenes estaban vacíos cuando las vacas comenzaron el pastoreo como ha sido reportado previamente (Al-Marashdeh et al., 2016b, Gregorini et al., 2010). De forma consistente con el pH ruminal, la concentración total de AGV tendió a ser mayor en las vacas PM que las AM-PM, que podría explicarse por un rumen más estable en las vacas AM-PM producto del patrón de ingestión (proporción de vacas pastoreando más constante durante toda la sesión, Capítulo 3).

Si bien la concentración de los productos finales de la fermentación ruminal refleja la eficiencia del proceso fermentativo, la cantidad absorbida de nutrientes depende de la producción total de estos productos finales. La mayor estabilidad en el pH y concentración de amonio en el rumen durante el día en las vacas que comieron ensilaje de maíz en 2 comidas (AM-PM), se reflejó en los menores contenidos totales de amonio y AGV respecto de las vacas que se les ofreció el ensilaje de maíz de una sola vez. Las vacas AM-PM presentaron menor fracción líquida respecto a los otros tratamientos que estuvo asociada con un mayor tamaño del pool de MS y a una mayor capacidad buffer del rumen como ha sido planteado por Chilibroste et al., (2001). Adicionalmente, el grupo AM-PM presentó un mayor consumo de MS lo que puede haber provocado una mayor tasa de pasaje. El peso de la fracción sólida y de MO fue mayor para las vacas PM y el aumento a lo largo del día de ambas variables fue consistente con el tratamiento alimenticio impuesto (pastura + ensilaje), y refleja la dinámica de llenado ruminal reportada previamente (Gregorini et al., 2010, 2009b, Chilibroste et al., 2001). El patrón del pool de amonio es resultado del patrón de ingestión acumulativo de forraje dado que hace un pico para todos los tratamientos al final de la sesión de pastoreo. Finalmente, la reducción del tamaño del pool de AGV en las vacas AM-PM podría estar relacionada con el menor tamaño de la fracción liquida como reflejo de una condición del rumen más estable durante el día. Es probable que las vacas AM-PM experimentaran una mejor sincronía de nutrientes (Kolver et al., 1998) y una mejor eficiencia a nivel ruminal (Trevaskis et al., 2004), lo que es consistente con la tendencia de encontrar mayor LCG y CC de las vacas en el tratamiento AM-PM.

6. CONCLUSIONES

Restringir el tiempo de acceso al pastoreo de 8 a 4 h disminuyó el CMS y la producción de leche. Dentro de los tratamientos de 4 h, las vacas que comenzaron la sesión de pastoreo a las 11:00 h tendieron a una tasa de consumo más alta y produjeron más proteína en leche que las vacas que comenzaron la sesión de pastoreo más temprano en la mañana.

Los resultados de este estudio tienen una fuerte implicancia práctica ya que el manejo alternativo de los mismos recursos (pastura y animales) puede resultar en un beneficio económico. Además de las ventajas que tiene el pastoreo restringido en los animales, también podría disminuir los efectos negativos del animal sobre la pastura especialmente cuando ésta es más propensa al daño por pisoteo (barro o exceso humedad).

En las condiciones del Capítulo 3, las vacas que recibieron ensilaje de maíz en dos comidas aumentaron el CMS, rendimiento de LCG y CC en comparación con las vacas que lo recibieron antes del pastoreo. Estas respuestas resultaron en un mayor consumo de forraje y total y en una mejor sincronización de nutrientes mediada por cambios en el comportamiento ingestivo de los animales.

El momento de suministro del ensilaje de maíz con respecto a la sesión de pastoreo impactó en el llenado del rumen y la cinética de fermentación. Fraccionar el ensilaje de maíz en dos comidas (antes y después del pastoreo) mantiene condiciones ruminales más estables basadas en el pH y en los pooles y concentraciones de amonio y AGV a nivel ruminal. Estas condiciones del rumen más estables no afectaron el patrón de degradación del heno de alfalfa, pero es el principal factor que explica el mayor CMS observado en este tratamiento.

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