Interaction between herbage mass and time of herbage allocation modifies milk production, grazing behaviour and nitrogen partitioning of dairy cows

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\textbf{Abstract.} The objective of the present study was to evaluate the interaction effects between herbage mass and time of herbage allocation on milk production, grazing behaviour and nitrogen partitioning in lactating dairy cows. Forty-four Holstein Friesian cows were grouped according to milk production (24.7 ± 2.8 kg), bodyweight (580.6 ± 51.7 kg), days in milk (74 ± 17.1) and body condition score (3.1 ± 0.3), and then assigned randomly to one of four treatments: (1) L-AM: access to new herbage allocation after morning milking with herbage mass of 2000 kg DM/ha, (2) L-PM: access to new herbage allocation after afternoon milking with herbage mass of 2000 kg DM/ha, (3) M-AM: access to new herbage allocation after morning milking with herbage mass of 3000 kg DM/ha, and (4) M-PM: access to new herbage allocation after afternoon milking with herbage mass of 3000 kg DM/ha. All cows received a daily low herbage allowance of 21 kg DM measured above ground level, 3.0 kg DM of grass silage and 3.5 kg DM of concentrate. Herbage intake was similar between treatments, averaging 8.3 kg DM/day (\(P > 0.05\)). Total grazing time was lower for M-PM compared with other treatments (\(P < 0.01\)). Milk production was greater for M-AM and M-PM compared with L-PM (\(P < 0.05\)). Urea in milk and plasma were greater for L-AM than L-PM and M-PM (\(P < 0.01\)). Similarly, rumen ammonia was greater for L-AM compared with M-PM and M-AM (\(P < 0.05\)). Nitrogen intake was 13.6% greater for L-AM than L-PM, and 17.5% greater for L-AM than M-PM (\(P < 0.01\)). Nitrogen use efficiency was 22.1% greater for M-PM than L-AM, and 11.8% greater for M-PM than L-PM (\(P < 0.01\)). In conclusion, the best management combination was observed when a medium herbage mass was delivered in the afternoon, maintaining a low nitrogen intake, low urinary nitrogen excretion and high milk production.

\textbf{Additional keywords:} autumn grazing, nitrogen use efficiency, perennial pasture, urine nitrogen.

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\textbf{Introduction}

In recent years there has been an increased demand to reduce the environmental footprint of pastoral livestock production, making it necessary to adjust social and environmental constraints of dairy farming, yet still achieve production and financial goals (Gregorini \textit{et al.} 2016; Beukes \textit{et al.} 2017). This challenge is associated with the high nitrogen (N) excretion, ranging between 60 and 90% of the N intake (Flachowsky and Lebzien 2006). The majority of N is excreted in the urine, with 50–90% of excreted N being ureic-N, which is more readily available for enzymatic action of bacteria present in faeces and soil (Bussink and Oenema 1998; Selbie \textit{et al.} 2015), and is readily available for leaching and volatilisation, which contribute to greenhouse effects (Tamminga 1992). The low N use efficiency (NUE; 17–35%) of grazing diary systems is attributed to the high N intake, high soluble protein in the herbage and an imbalance in the supplies of water-soluble carbohydrates (WSC) and crude protein (CP) (Hoekstra \textit{et al.} 2007; Pacheco and Waghorn 2008).

Despite the issues described above, grazing is the cheapest source of food for dairy cows (Pulido \textit{et al.} 2010). Therefore, it is fundamental to evaluate strategies that maintain herbage as the main diet component in order to achieve improvements. A viable strategy to improve NUE is the combination between carbohydrate supplementation and offering of herbage during the afternoon (Keim and Anrique 2011; Gregorini 2012). The latter is due to the increasing concentration of WSC throughout the day in response to sugar accumulation during photosynthetic activity, in contrast to CP concentration, which is usually highest.
in the morning (Delagarde et al. 2000; Vibart et al. 2017). In addition, the herbage with the highest nutritive value in the afternoon is associated with the best dry matter (DM) concentration, digestibility and fibre dilution (Vibart et al. 2017). Several studies have evaluated the timing of herbage allocation, but without concordance in their effects on NUE (Abrahamse et al. 2009; Vibart et al. 2017). Furthermore, these diurnal fluctuations in the chemical composition of herbage have been associated with changes in temporal patterns of herbage intake in grazing dairy cows, with increases in the herbage intake when cows received a new herbage allocation in the afternoon, coinciding with the highest DM andWSC concentration (Orr et al. 2001; Vibart et al. 2017). The total DM intake (DMI) has been shown to be similar between dairy cows receiving new fresh herbage in the morning or afternoon under autumn (Pulido et al. 2015; Vibart et al. 2017), spring (Orr et al. 2001) and summer (Abrahamse et al. 2009) conditions.

Pre-grazing herbage mass (HM) appears to be a key factor in determining the herbage DMI and animal performance due to its effect on grazing behaviour and herbage chemical composition (Wims et al. 2010; Pérez-Prieto and Delagarde 2012). In herbage evaluated at the ground level, it has been observed that the CP concentration is lower at higher HM (Pérez-Prieto et al. 2013), which suggests that HM could alter the N flow through the animal, and thereby change N partitioning into milk, urine and faeces. However, to the best of our knowledge, there are no studies evaluating the HM and timing of herbage allocation effects on milk production and N excretion. Our hypothesis is that dairy cows receiving a medium HM in the afternoon have a greater milk production and NUE, and lower urinary N excretion compared with cows receiving a low HM in the morning, in response to changes in grazing behaviour, herbage DMI and rumen fermentation. The aim of this study was to evaluate the effect of timing of herbage allocation and HM on milk production, grazing behaviour and N excretion of lactating dairy cows during autumn.

Materials and methods

All procedures regarding animal handling and treatments described in this experiment were approved by the Animal Welfare Committee of the Universidad Austral de Chile.

The experiment was carried out between 5 May to 1 July 2016 at Universidad Austral de Chile’s Agricultural Research Station (latitude 39°47’S and longitude 73°14’W, annual rainfall 2500 mm).

Cows, experimental design and treatments

Forty-Four Holstein Friesian cows, including four fistulated cows, were grouped according to milk production (24.7 ± 2.8 kg), bodyweight (BW; 580.6 ± 51.7 kg), days in milk (74 ± 17.1) and body condition score (BCS; 3.1 ± 0.3). The groups were randomly allocated to one of four treatments: (1) L-AM: access to new herbage allocation after morning milking with HM of 2000 kg DM/ha, (2) L-PM: access to new herbage allocation after afternoon milking with HM of 2000 kg DM/ha, (3) M-AM: access to new herbage allocation after morning milking with HM of 3000 kg DM/ha, and (4) M-PM: access to new herbage allocation after afternoon milking with HM of 3000 kg DM/ha. The four ruminally fistulated dairy cows were allocated to one of four treatments in a Latin square design, staying on each treatment for 14 days, comprising a 13-day diet adaptation period and a 1-day recording period to allow measurement of rumen metabolism.

All groups were strip grazed with a daily low herbage allowance of 21 kg DM/cow measured above ground level, offered at 0900 hours (L-AM and M-AM treatments) or 1600 hours (L-PM and M-PM treatments). In addition, all cows were supplemented with 3.5 kg DM/day of concentrate, which was divided into equal amounts during both milking times (0700 hours and 1400 hours). The concentrate comprised (% on DM basis) 49.3 corn, 11.5 soybean meal, 30.0 beet pulp, 4.6 beet molasses and 4.5 mineral mix. Furthermore, all cows received 3.0 kg DM/day of grass silage, which was divided into equal amounts after both milking times (0800 hours and 1500 hours) in individual feeding pens. Silage and concentrate refusal was measured individually for each cow. The first 14 days of the experiment were considered the adaptation period to the treatments.

Herbage and grazing management

Grazing took place on 20.3 ha of permanent herbage dominated by perennial ryegrass (Lolium perenne L.), which was subdivided into six paddocks. The sward was established 4 years earlier and subjected to strip-grazing management. All treatments were grazed in the same paddock, but were separated by an electric fence according to correspondent HM. All cows were given access to new fresh herbage after each milking according to treatment.

The area to be grazed each day was adjusted by herbage allowance and pre-grazing HM. The pre-grazing HM (kg DM/ha, above ground level) was estimated three times per week using a rising plate meter (Ashgrove Plate Meter, Hamilton, New Zealand). Each estimation considered 100 compressed sward height measurements by walking through the herbage in a ‘W’ pattern. Then, using a specific equation for autumn grasslands of southern Chile (Canseco et al. 2007), compressed height data (cm) was transformed into kg DM/ha. Post-grazing HM was estimated using the same methodology. The equation used is as follows:

\[ Y = 120X + 350 \]

\[ R^2 = 0.74 \]

where Y is HM expressed in kg DM/ha, and X is average compressed height.

To allow a difference of 1000 kg DM/ha between low and medium HM treatments, 1 month before the start of the experiment, all paddocks were grazed successively by non-experimental cows. Every time that herbage in the paddock grew up to 2000 kg DM/ha, then 60% of each paddock was again grazed by non-experimental cows, and then used for L-AM and L-PM treatments during the experiment. For the remaining 40% of each paddock, they were again grazed when herbage grew to 3000 kg DM/ha, and then used by M-AM and M-PM treatments during the experiment.

Herbage and supplement sampling and analyses

Herbage samples were collected once a week at 0900 hours for L-AM and M-AM treatments, and 1600 hours for L-PM and
M-PM treatments. Each weekly herbage sample was composed by three sampling days (Monday, Wednesday and Friday). Herbage samples were collected using the hand-plucking technique (Pulido and Leaver 2001), with samples immediately frozen for subsequent analysis. Supplement samples (silage and concentrate) were collected and immediately frozen three times during the experiment. All samples were freeze-dried, and before chemical analysis, the samples were ground through a 1-mm screen (Willey Mill; 158 Arthur H. Thomas, Philadelphia, PA, USA), and analysed for DM, CP, acid detergent fibre and ash according to AOAC (1996). Neutral detergent fibre (NDF) was determined according to Van Soest et al. (1991). Metabolisable energy (ME) concentration of the herbage was estimated using the following equation:

$$ME = 0.279 + 0.0325 \times \% \text{D-value} \quad \text{(Garrido and Mann 1981)}.$$  

D-value was estimated in vitro using the equation reported by Tilley and Terry (1963).

**DMI and grazing behaviour**

Herbage DMI was estimated from energy requirements of the cows and ME concentration of the grass and supplements using the follow equation:

$$\text{Herbage DMI (kg/day)} = \left( ME_{\text{r}} + ME_{\text{mg}} + ME_{\text{fwe}} + ME_{\text{g}} \right) - \left( \text{Conc ME + silage ME} \right) \over \text{Herbage ME}$$

where $ME_{\text{r}}, ME_{\text{mg}}, ME_{\text{fwe}}$ and $ME_{\text{g}}$ are the ME requirements for maintenance, milk yield, change in liveweight and gestation, respectively (Baker 2004).

Grazing behaviour was evaluated twice throughout the experiment (Days 22 and 46) over 24 continuous hours. Grazing activities (grazing, ruminating and idling) were recorded every 10 min during daylight (0800–1800 hours) and every 15 min at night (1800–0800 hours). Each treatment had one observer, so that observations were simultaneous.

**Milk production, BW and BCS**

Daily milk production was recorded with an automated system (MPC580; DeLaval, Tumba, Sweden). Milk samples were collected during morning and afternoon milking on Days 19, 33, 40 and 47 of the experiment. These samples were used to estimate the protein, fat and urea in milk by infrared spectroscopy (Milko-scan, System 4300; Foss Electric, Hillerod, Denmark).

Daily BW was recorded after both milkings with an automated system in the milking parlour. In addition, BCS was recorded once per week after morning milking by one trained observer using a five-point scale (Ferguson et al. 1994).

**Urine and blood parameters**

Spot urine samples were collected on Days 19 and 47 of the experiment after the morning and afternoon milking. Approximately 10 mL of urine was collected during voluntary excretion or manual stimulation. After collection, urine samples were acidified with sulfuric acid (10% v/v) to minimise volatilisation and frozen at −20°C for subsequent chemical analysis. Before chemical analysis, the samples were thawed and analysed for estimation of purine derivatives (allantoin, uric acid) and creatinine by high-performance liquid chromatography (Diez et al. 1992). The microbial N production (g/day) was calculated from the purine derivatives : creatinine ratio using equations derived by Chen and Ørskov (2004). The efficiency of microbial protein synthesis in the rumen was calculated as g microbial N production synthesised per kg of rumen degradable organic matter.

Coccygeal blood samples were obtained on Days 14, 35 and 49 of the experiment. Blood samples were collected after afternoon milking using vacutainers containing lithium heparin. Once the samples were collected, plasma was separated by centrifugation at 800g for 10 min and then frozen at −20°C for subsequent analysis. Plasma was used to measure the concentration of β-hydroxybutyrate (Randub; Randox Laboratories, Crumlin, County Antrim, Northern Ireland) and urea (glutamate dehydrogenase, HUMAN, Wiesbaden, Germany) by Wiener Metrolab 2300® auto-analysers (Wiener Laboratory, Rosario, Argentina).

**Rumen fermentation parameters**

Four cattle fitted with rumen fistula were utilised to determine the rumen pH, ammonia (NH₃) and volatile fatty acids (VFA) concentration using a 4 × 4 Latin square design, with four cows, four periods and four treatments. Once each period was finished, the fistulated cows were allocated to a different treatment.

The ruminal samples were collected from three sites within the rumen (cranial, ventral and caudal) at 0730, 0930, 1300, 1600, 1900, 2100, 0000 and 0300 hours during Days 15, 29, 43 and 57 of the experiment. Immediately after collection of rumen fluid, pH was measured by glass electrode (Model HI98127; Hanna Instruments, Woonsocket, RI, USA). After pH was recorded, the ruminal fluid samples (from each ruminal site) were bulked and subdivided into two samples; one subsample of 20 mL was stored at −20°C for subsequent analysis. An aliquot of 4 mL was diluted with formic acid (4 : 1 ratio), and the rumen concentration of VFA was determined by gas chromatography (Shimadzu GC-2010 Plus High-end gas chromatography, equipped with gas chromatography capillary column, SGE, BP21 (FFAP); Shimadzu Corporation, Kyoto, Japan). A second subsample of 10 mL was acidified with 0.2 mL of 50% trichloroacetic acid solution to estimate rumen NH₃ concentration by spectrophotometry (Spectronic Genesys 5® spectrophotometer; Milton Roy, Iyivald, PA, USA), as described by Bal et al. (2000).

**Nitrogen partitioning**

N partitioning was estimated during Weeks 3 and 7 of the experiment, using blood, herbage, supplements and milk samples collected at this time. The equations used were:

$$N \text{ intake} = [(\text{herbage DMI} \times \% \text{N in herbage / 100}) + \text{(concentrate DMI} \times \% \text{N in concentrate / 100}) + \text{(grass silage DMI} \times \% \text{N in grass silage / 100})]; \text{Milk N (g N/day)} = (\text{milk yield} \times \% \text{N in milk / 100}) \times \text{BW (kg)} \times \text{plasma urea N (g/L)} \quad \text{(Kohn et al. 2005)}.$$  

**Statistical analyses**

Milk production, milk composition, grazing behaviour, blood, N partitioning and urinary metabolites were analysed as repeated
measures in time using the MIXED procedure of SAS (PROC MIXED; SAS Institute, Cary, NC, USA). The model included the fixed effect of treatment, random effect of cows, day of sampling as repeated measure, and interaction between treatment and day of sampling. Milk production, BW and body condition score were analysed including the pre-experimental period as a covariate. The experimental unit was the cow. DMI and change in BW and BCS were analysed using a mixed model procedure (PROC MIXED; SAS). The model included the fixed effects of treatment and the random effect of cows.

Chemical composition of pasture, HM (pre- and post-grazing) and sward height (pre- and post-grazing) were analysed using a mixed model procedure (PROC MIXED; SAS). The model included the fixed effects of treatment, day of sampling, and their interaction, and the random effect of paddock. Day of sampling and interaction were not significant, and were subsequently dropped from the model. The experimental unit was the paddock.

Rumen fermentation parameters (pH, NH$_3$-N and VFA) from fistulated cows were analysed by repeated measures ANOVA using the mixed model procedure (PROC MIXED; SAS). The model included the fixed effects of treatment and period, random effect of cows, time of sampling as repeated measure, and interaction between treatment and time of sampling.

Comparison between treatments was carried out using the Tukey test. Results were considered significant at $P < 0.05$, and tendency at $P < 0.1$

**Results**

**Chemical composition of herbage and supplements**

The chemical composition of herbage and supplements is presented in Table 1. DM concentration of the herbage was different among treatments ($P < 0.05$), being 20% lower for L-AM than L-PM, and 19% lower for L-AM than M-PM. The herbage concentration of CP and soluble protein were greater ($P < 0.05$) for L-AM than other treatments. The herbage WSC concentration was 30% lower for L-AM than L-PM, and 35% lower for L-AM than M-PM. Metabolisable energy and NDF concentration of pasture were not affected by treatments, averaging 11.6 MJ ME/kg DM and 49.0% respectively. Digestibility of pasture was similar among treatments, averaging 77.0%.

**HM and DMI**

The results of HM and DMI grazing behaviour are presented in Table 2. Pre- and post-grazing HM were greater ($P < 0.05$) for M-AM and M-PM compared with L-AM and L-PM. Herbage and total DMI were not affected by treatments ($P > 0.05$), averaging 8.3 and 14.8 kg DM/cow respectively. Despite similar herbage DMI among treatments, the herbage WSC intake was affected by treatments, being greater for M-PM and lower for L-AM than other treatments ($P < 0.05$). Crude protein intake was greater for M-AM compared with L-PM and M-PM ($P < 0.05$). However, ME intake was similar among treatments ($P > 0.05$), averaging 177 MJ/cow.

**Grazing behaviour**

The results of grazing behaviour are presented in Table 3. Total grazing time was different between treatments ($P < 0.05$), being lower for M-PM compared with other treatments. Grazing time between morning and afternoon milking (0700–1400 hours) was longer ($P < 0.01$) for L-AM and M-AM than L-PM and M-PM, and shorter for M-PM than other treatments. Grazing time between afternoon and morning milking (1600–0645 hours) was lower ($P < 0.01$) for L-AM and M-AM than for L-PM and M-PM. Total rumination and idling time were not affected by treatments ($P > 0.05$), averaging 411 and 603 min/day respectively.

**Milk production, BW and BCS**

The results of milk production, milk composition, milk solids (fat and protein), and change in BW and BCS are presented in Table 4. Milk production was different among treatments ($P < 0.05$), being 7.6% greater for M-AM than L-PM, and 4.8% greater for M-PM than L-PM, but similar between L-AM, M-AM and M-PM. Protein and fat in milk were not affected by treatments, averaging 3.2% and 3.9% respectively. However, urea concentration in milk differed among treatments ($P < 0.05$),

**Table 1. Chemical composition of herbage and supplements offered to dairy cows during the experiment**

<table>
<thead>
<tr>
<th></th>
<th>L-AM</th>
<th>L-PM</th>
<th>M-AM</th>
<th>M-PM</th>
<th>s.e.</th>
<th>$P$-value</th>
<th>Grass silage</th>
<th>Supplements</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (%)</td>
<td>11.5b</td>
<td>14.3a</td>
<td>12.1ab</td>
<td>14.2a</td>
<td>0.57</td>
<td>&lt;0.01</td>
<td>37.3</td>
<td>2.75</td>
<td>86.4</td>
</tr>
<tr>
<td>CP (%)</td>
<td>31.4a</td>
<td>27.6b</td>
<td>26.4b</td>
<td>22.7c</td>
<td>0.67</td>
<td>&lt;0.01</td>
<td>14.9</td>
<td>1.35</td>
<td>11.5</td>
</tr>
<tr>
<td>SP (%)</td>
<td>12.8a</td>
<td>10.6b</td>
<td>10.9b</td>
<td>8.9c</td>
<td>0.48</td>
<td>&lt;0.01</td>
<td>49.5</td>
<td>2.85</td>
<td>32.3</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>49.5b</td>
<td>48.1a</td>
<td>50.5b</td>
<td>48.1b</td>
<td>1.92</td>
<td>&lt;0.01</td>
<td>46.6</td>
<td>2.85</td>
<td>32.3</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>21.8a</td>
<td>21.6b</td>
<td>23.4b</td>
<td>23.4b</td>
<td>0.52</td>
<td>&lt;0.01</td>
<td>28.9</td>
<td>1.45</td>
<td>15.5</td>
</tr>
<tr>
<td>ME (%)</td>
<td>11.7b</td>
<td>14.8a</td>
<td>12.1ab</td>
<td>14.2a</td>
<td>0.57</td>
<td>&lt;0.01</td>
<td>11.81</td>
<td>0.46</td>
<td>13.19</td>
</tr>
<tr>
<td>WSC (%)</td>
<td>5.7b</td>
<td>5.2a</td>
<td>7.1ab</td>
<td>7.6a</td>
<td>0.24</td>
<td>&lt;0.01</td>
<td>8.2</td>
<td>0.54</td>
<td>–</td>
</tr>
<tr>
<td>Digestibility (%)</td>
<td>77.7</td>
<td>78.0</td>
<td>76.1</td>
<td>76.2</td>
<td>0.83</td>
<td>0.25</td>
<td>77.4</td>
<td>0.92</td>
<td>86.5</td>
</tr>
<tr>
<td>pH</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>N-NH$_3$ (%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8.2</td>
</tr>
</tbody>
</table>
being 13% greater for L-AM compared with L-PM, 19.6% greater for L-AM than M-AM, and 21.7% greater for L-AM than M-PM. Milk solids were different among treatments ($P < 0.05$), being 21% greater for M-PM than L-PM. Change in BW and BCS was not affected by treatments ($P > 0.05$).

### Urine and blood parameters

Blood and urinary results are presented in Table 5. Plasma $\beta$-hydroxybutyrate concentration was greater for L-AM and M-AM compared with L-PM and M-PM ($P < 0.05$). Likewise, plasma urea concentration was greater for L-AM and M-AM than for L-PM and M-PM ($P < 0.05$). Urinary metabolites and synthesis of ruminal microbial protein were unaffected by treatment ($P > 0.05$).

### Rumen pH, ammonia and VFA

Rumen fermentation parameters are presented in Table 6. Ruminal concentration of N-NH$_3$ was 21% greater ($P < 0.05$) for L-AM than
M-AM and M-PM. Rumen pH was unaffected by treatment \((P > 0.05)\), averaging 6.1. Concentration of acetate, butyrate and propionate were similar among treatments, averaging 65.1, 12 and 18 mM/100 M, respectively. The total VFA and acetate : propionate ratio was unaffected by treatments \((P > 0.05)\), averaging 66.3 mM/day and 3.77 respectively.

**Nitrogen intake and excretion (milk, faeces and urine)**

The results of N partitioning are presented in Table 7. N intake differed among treatments \((P < 0.05)\), being 13.7% greater for L-AM than L-PM, and 17.7% greater for L-AM than M-PM. Milk N was similar among treatments, averaging 113 g N/day. Nitrogen use efficiency (milk N/N intake) was greater for M-PM compared with L-AM and L-PM, and lower for L-AM compared with other treatments \((P < 0.05)\). Urinary N excretion was 21.1% lower for L-PM compared with L-AM, 25.5% lower for M-PM than L-AM, and 23.7% lower for M-PM compared with M-AM. Urinary N excretion (g N) per unit of milk production (kg milk) was 28.9% lower for M-PM compared with L-AM \((P < 0.05)\), and similar between M-PM, M-AM and L-PM.
Discussion

This research evaluated the relationship between HM and time of pasture allocation in dairy cows receiving a moderate supplementation during a short-term grazing experiment. Several works have evaluated the effects of time of herbage allocation (Gregorini 2012; Pulido et al. 2015; Vibart et al. 2017) and pre-grazing HM (Wales et al. 1999; Pérez-Prieto et al. 2013; Muñoz et al. 2016) on milk production, grazing behaviour and N partitioning of grazing dairy cows; however, to the best of our knowledge, there are no studies evaluating the interaction between both factors.

Grazing management, DMI and grazing behaviour

The difference between medium and low HM was 1131 kg DM/ha, which was greater than the minimum difference expected (1000 kg DM/ha) to highlight the effects of HM on milk production, grazing behaviour and N partitioning.

Similar herbage DMI between treatments suggests that the time of herbage allocation, herbage allowance used and the difference in the HM between treatments were not enough to modify intake. In this sense, it can be suggested that herbage allowance of 21 kg DM and 1132 kg of DM/ha of the difference between medium and low HM appears to be too low to increase the unavailable sward fraction (herbage available under 2.5 cm) in treatments receiving a low HM compared with those treatments receiving a medium HM. The results of Pérez-Prieto et al. (2013) support our assumption, where a greater difference between medium and low HM than in the current experiment meant that the unavailable sward fraction was greater for low than medium HM, and a reduction of 41% in the real herbage allowance for low compared with medium HM, which was reflected in a lower DMI. In this way, the lack of difference in the herbage DMI in the current experiment indicates that cows in the L-AM and L-PM treatments were able to graze more efficiently the partially available herbage fraction (between 2.5 to 5.0 cm above ground level) than the M-AM and M-PM cows, which can be explained by the 0.9-cm lower post-grazing sward height for L-AM and L-PM compared with M-AM and M-PM (3.6 and 4.5 cm respectively). In contrast, several authors (Pulido et al. 2015; Vibart et al. 2017) have found a similar herbage DMI between cows receiving a new herbage allocation in the morning or afternoon. In addition, the moderate supplementation level could disguise the potential effects of time of herbage allocation and HM on DMI due to a high substitution rate. Finally, the methodology used to estimate total DMI could not be adequate to highlight the difference in the total DMI, because it assumes that all ME intake is fully utilised by the cows. Therefore, in combination, these results suggest that the difference in HM may not have been enough (1132 kg of DM/ha) to affect the herbage DMI in grazing dairy cows receiving a low herbage allowance of 21 kg DM and moderate supplementation.

Lower grazing time for M-PM compared with other treatments suggests that combination between HM and time of herbage allocation allowed the daily grazing pattern to change. The longer grazing time for L-AM, L-PM and M-AM indicated that grazing conditions were more constraining for animals to get the grass, spending more time eating than other activities, which can be related to the available HM for L-AM and L-PM, and herbage chemical composition for M-AM. According to Pérez-Prieto et al. (2013), longer grazing time at low HM occurred as a compensatory response to the reduction in sward height. Although cows in M-AM grazed with a medium HM, the grazing time was similar to L-AM and L-PM, which indicates that medium HM delivered in the morning produced adverse grazing conditions, possibly connected to the low DM concentration in the herbage. It has been observed that internal water in the herbage dilutes the feed intake in dairy cows, because it cannot be swallowed immediately and must be masticated, limiting the DMI eating rate (Cabrera Estrada et al. 2004) and increasing eating time. Cabrera Estrada et al. (2004) found that grazing time tended to be longer for herbage with 12% than 16% DM, suggesting that longer grazing time in M-AM could be associated with its numerical 2.1 unit of % lower DM concentration than M-PM. However, several works have found no difference in grazing time between cows receiving a new herbage allowance in the morning or afternoon (Gregorini et al. 2008; Abrahamse et al. 2009; Pulido et al. 2015; Vibart et al. 2017). Furthermore, the herbage DM concentration in these studies ranged from 15.1% to 32.0% from low to medium HM, which suggests that time of herbage allocation can modify the total grazing time in herbage with low DM concentration.

Milk production and composition

A greater milk production was expected in cows receiving a medium HM in the afternoon than those receiving low HM in the morning; however, milk production was similar between these treatments. This result suggests that greater WSC intake for M-PM and M-AM than L-AM was not enough to produce a change in the energy intake, which is considered a factor limiting milk production (Ruiz-Albarrán et al. 2012).

In contrast, the greater milk production for M-AM and M-PM compared with L-PM can be associated with its numerically greater herbage DMI (+1.4 and +1.1 kg DM respectively). Similar propionate concentration among treatments suggests that greater WSC intake for L-PM, M-PM and M-PM compared with L-AM was not enough to modify the molar concentration of VFA. Similar results were reported by Pérez-Prieto et al. (2013), where cows receiving a medium HM had a greater milk production than cows receiving a low HM. Therefore, changes in milk production seem to be more associated with the available pre-grazing HM than time of herbage allocation.

Greater milk production has been associated with greater synthesis of rumen microbial protein (Bargo et al. 2003); however, in our experiment, the greater milk production was not associated with greater synthesis of rumen microbial protein, suggesting that the use of two urine samples per day could be insufficient to catch the daily variation of purine derivatives excreted in urine and, thereby, synthesis of microbial protein.

Similar milk protein concentration between treatments can be explained by the similar energy intake between treatments (177 MJ of ME/day), as reported by Coulon and Rémont (1991). Similar milk fat can be associated with the similar NDF intake and acetate : propionate ratio between treatments (Walker et al. 2004).
The lower milk urea for M-AM and M-PM compared with L-AM suggests a more efficient utilisation of dietary protein and ruminal NH₃. The above described is supported by the lower ruminal NH₃ concentration in M-AM and M-PM than L-AM, being a consequence of the lower CP intake and greater WSC intake for both treatments compared with L-AM. This result indicates that M-AM and M-PM treatments were more efficient than L-AM to convert the dietary N into microbial protein, because they produced similar rumen microbial protein with lower N intake and greater WSC intake. A similar result was observed by Carruthers et al. (1996), where extra WSC reduced ruminal NH₃ and milk urea concentrations. In addition, several authors have found greater ruminal N-NH₃ concentration in cows where rumen degradable protein was greater than fermentable energy (Castillo et al. 2001; Külling et al. 2001).

However, the low milk urea and low rumen NH₃ for H-AM was not reflected in a low plasma urea concentration, which could be associated with the blood sampling used in the current experiment. Blood samples were collected after afternoon milking, suggesting that the high plasma urea concentration for H-AM just showed the normal peak of plasma urea (4 h after feeding) (Noro et al. 2012).

Rumen VFA concentration

Similar propionate and butyrate concentration among treatments suggests that greater WSC intake for L-PM, M-AM and M-PM compared with L-AM was not enough to modify their molar concentration. Similar results were reported by Taweel et al. (2005), where greater WSC concentration in the pasture was not enough to modify propionate and butyrate concentration. The lack of concordance can be explained by the slight correlation ($r = 0.49$) between milk production and propionate concentration (Seymour et al. 2005). In addition, Seymour et al. (2005) reported a high correlation between milk production and DMI ($r = 0.83$), supporting our results. The similar acetate concentration between treatments can be explained by the similar NDF intake between treatments (Walker et al. 2004; Abrahamsen et al. 2009). Therefore, our results indicate that combination between pre-grazing HM and time of pasture allocation were not enough to modify the fermentation VFA pathways in the rumen.

N intake and N excretion (milk, faeces and urine)

The greater N intake for L-AM than L-PM and M-PM is mainly explained by its greater CP concentration of the herbage, producing a greater CP intake per kg of herbage DMI. Similarly, Flachowsky and Lebzien (2006) found that N excess in the feeding increased N excretion per animal and per food of animal origin, supporting our results. This result suggests that time of herbage allocation was more important than HM regarding N intake, which can be associated with its effect on grazing behaviour and chemical composition of herbage. In this sense, cows in L-PM and M-PM spent 39.6% and 30.8% more time grazing in the afternoon compared with L-AM respectively. During the afternoon, herbage dominated by grasses are characterised as having a greater WSC and lower CP concentration than in the morning, in response to sugar accumulation during photosynthetic activity (Rutter et al. 2004; Lund et al. 2008; Vibart et al. 2017). Therefore, the lower N intake for L-PM and M-PM was in response to greater herbage DMI in the time of day when herbage had a greater WSC and lower CP concentration.

Similar milk N between treatments indicates that all extra N ingested by cows grazing L-AM compared with L-PM and M-PM was excreted through urine and faeces. A similar result was reported by Mulligan et al. (2004), where greater N intake was not reflected in greater milk N, instead increasing urinary N excretion. The greater NUE for M-PM and L-PM compared with L-AM occurred in response to its lower N intake. According to Pacheco and Waghorn (2008), the NUE can be improved by increasing animal performance or by reducing the dietary intake of CP. Similar results were reported in other works (Burke et al. 2008; Whelan et al. 2012), where the higher NUE was associated with lower N intake.

Urine N excretion was greater for L-AM than M-PM and L-PM, in response to its highest N intake. The CP intake for L-AM exceeded the recommended CP requirements for cows at this stage of lactation by 36.0% (2.47 vs 3.36 kg CP/day), while treatments L-PM and M-PM exceeded only in 21.3% (2.39 vs 2.90 kg CP/day) and 11.7% (2.47 vs 2.76 kg CP/day) (NRC, 2001) respectively, showing that the greater N intake was not used for production in L-AM treatment; instead, it was eliminated through urine, as was observed in our experiments. Several studies have found that urinary N excretion is linearly related to N intake (Tas et al. 2006; Hoekstra et al. 2007; Higgs et al. 2012), whereas faecal and milk N are scarcely modified when dietary N intake is increased (Pacheco and Waghorn 2008). In contrast, the lower urinary N excretion for M-PM compared with L-AM allowed a reduction by 28.9% in the urinary N excretion per kg of milk production.

This short-term grazing experiment shows that combination between HM and time of herbage allocation modified milk production, grazing behaviour, rumen function and N excretion in dairy cows; however, there is no evidence that the same results could occur along the entire grazing season.

Conclusion

Simple changes in the daily grazing management, such as time of herbage allocation and pre-grazing HM, were enough to modify the chemical composition of pasture, milk production and N excretion. In this way, it was possible to reduce urinary N excretion and maintain high milk production in grazing dairy cows receiving a medium HM in the afternoon.

Conflicts of interest

The authors declare no conflicts of interest.

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