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RESEARCH ARTICLE

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The timing of pasture allocation and grass silage supplementation affect pasture intake, milk production and nitrogen partitioning of dairy cows

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Abstract

Aim of study: To evaluate the effect of time of pasture allocation (PA) and grass silage allocation on dry matter (DM) intake, grazing behaviour, milk production, rumen function and N partitioning of grazing dairy cows.

Area of study: Valdivia, Chile

Material and methods: Forty-five Holstein-Friesian cows were selected from the Austral Agricultural Research Station at the Universidad Austral de Chile. Cows were allocated to one of three treatments: MPA: 75% of PA and 25% of the silage allowance allocated in the morning; BPA: 50% of PA and silage allowance allocated in the morning; APA: 25% of the PA and 75% of the silage allowance allocated in the morning. All treatments received the complement of pasture and silage allowance in the afternoon. Cows received a daily PA of 21 kg dry DM, 3 kg DM of grass silage and 3.5 kg DM of concentrate.

Main results: Grazing time was not affected by treatments, however, grazing time between afternoon-morning milking was longer for APA. DM intake and milk production were not modified by treatments, averaging 15.6 kg DM/cow and 22.7 kg milk/d, respectively. Rumen propionate was greater for BPA than APA (18.8 and 17.7 mmol/100 mol, respectively). N intake and N excretion throughout milk, urine and feces were not modified by treatments, averaging 458, 119, 195 and 144 g N/d, respectively.

Research highlights: The combination of time of pasture and grass silage allocation is not an adequate strategy to modify pasture intake, milk production and N excretion in dairy cows.

Additional key words: grazing behavior; grazing management; milk quality; nitrogen use efficiency; rumen function.

Abbreviations used: ADF (acid detergent fiber), BCS (body condition score), BW (body weight), CP (crude protein), DM (dry matter), DMI (dry matter intake), ME (metabolizable energy), NDF (neutral detergent fiber), NUE (nitrogen use efficiency), PA (pasture allowance), VFA (volatile fatty acids), WSC (water soluble carbohydrates),

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Introduction

As in most temperate climates, the predominant dairy systems are those that base their feeding on permanent pastures used mainly by grazing during the spring, summer, and autumn, because they are more cost effective than indoor system based on concentrate feeding (Dillon *et al.*, 2005). However, during autumn, the low dry matter (DM), low water-soluble carbohydrates (WSC) and high crude protein (CP) content limit milk production of autumn calving dairy cows due to low pasture DM intake (Pulido *et al.*, 2010; Ruiz-Albarrán *et al.*, 2016).

In addition, environmental pollution occurs as a consequence of N intake from pasture exceeding N cow requirements. Therefore, all N that is not used for maintenance and production purposes is excreted through urine and feces, contributing to environmental pollution by emissions of nitrous oxide and ammonia (Selbie *et al.*, 2015).

Supplementation of dairy cows on pasture is a strategy to improve milk production in response to increasing total DM intake (DMI), supplying energy and protein at appropriate concentrations to cover animal requirements (Bargo *et al.*, 2003). Grass silage supplementation is amply used during the autumn season in Southern Chile to cover pasture deficit and maintain a high DMI and milk production (Morales *et al.*, 2014; Ruiz-Albarrán *et al.*, 2016). However, effects of supplementation on DMI and milk production not only depend on composition and amount of supplement offered, but also on the timing of supplementation (Chilibroste *et al.*, 2008). The most important grazing bout in the day (morning and afternoon) can be modified by strategic supplementation at these times and thereby, pasture DMI, milk production (Sheahan *et al.*, 2013; Al-Marashdeh *et al.*, 2016b) and nutrient flow throughout day can be modified (Gregorini *et al.*, 2010). However, there is not a consensus as to its effect on pasture DMI, grazing behavior and milk production (Sheahan *et al.*, 2013; Al-Marashdeh *et al.*, 2016a; Mattiauda *et al.*, 2018).

Time of pasture allocation is another strategy to modify pasture DMI, milk production and N excretion: In comparison to pasture allocation in the morning, afternoon allocation is characterized by a greater WSC and DM and lower CP content of the pasture in response to sugar accumulation during photosynthesis and losses of moisture during the day (Delagarde *et al.*, 2000; Pulido *et al.*, 2015). This better chemical composition of afternoon pasture has been associated with greater WSC/CP intake and changes in rumen fermentation and grazing behavior (Abrahamse *et al.*, 2009; Pulido *et al.*, 2015). However, studies evaluating the time of pasture allocation have found that cows receiving a new pasture strip in the afternoon had similar (Abrahamse *et al.*, 2009) or tended to have greater milk production (Pulido *et al.*, 2015; Vibart *et al.*, 2017) and similar urine N excretion than cows receiving a morning new pasture strip (Vibart *et al.*, 2017).

Combining time of fresh pasture and supplementation allocation could be used as a strategy to alter milk production and N excretion due to their individual effects on pasture DMI, nutrient intake and rumen fermentation. Thus, it is possible to hypothesize that morning allocation of grass silage could reduce morning pasture DMI and increase afternoon DMI, where pasture is characterized by a better nutritional composition than that in the morning. The aim of this study was to evaluate the effect of time of fresh pasture and grass silage allocations on DMI, grazing behavior, milk production, rumen function and N partitioning of grazing dairy cows during autumn.

Material and methods

This experiment was carried out at the Austral Agricultural Research Station of the Universidad Austral de Chile (39°47′S, 73°13′W, Valdivia, Chile) over a period of 71 days (20th April to 30th June 2014), with 14 days of adaptation to the treatments and a 57-day measurement period. The climate is a typical cold weather Mediterranean, with a mean air temperature of 9.6° C (5.9° C - 13.3° C) and total precipitation of 830 mm during the experiment. The experimental procedures used in this study were approved by the Animal Welfare Committee of Universidad Austral de Chile.

Experimental design

Forty-five multiparous autumn calving Holstein-Friesian cows, including three rumen cannulated cows (milk production 24.5 ± 3.5 kg/d, body weight (BW) 516 ± 71 kg and days in milk 57.3 ± 10.4) were randomly allocated to one of three treatments: 1) MPA: 75% of the daily strip-pasture and 25% of the silage allowance were assigned in the morning; 2) BPA: 50% of the daily strip-pasture and 50% of the daily allowance were assigned in the morning; 3) APA: 25% of the daily strip-pasture and 75% of the daily silage allowance were assigned in the morning. All treatments received their remaining pasture-strip of fresh pasture and grass silage in the afternoon (Fig. 1).

The cows were strip-grazed with a daily fresh pasture allowance of 21 kg DM/cow (measured above ground level) offered after morning and afternoon milking according to the requirements of the respective treatments. All cows were offered 3.5 kg DM of cereal-based concentrate in two meals, 1.75 kg DM at each milking (07:00 and 14:00 h). All cows received a total of 3.0 kg DM of grass silage offered in feeding pens after milking times (08:00 h and 15:00 h) with the amount offered to each treatment differing in the morning and afternoon according to the requirements of the respective treatments. Concentrate was comprised of (on a % on DM basis) 49.3 maize, 11.5 soybean meal, 30.0 beet pulp, 4.6 beet molasses and 4.5 mineral mix.

The three rumen cannulated cows were allocated to one of three 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 for measurement of rumen fermentation parameters. Once each period finished, the cannulated cows changed treatment.

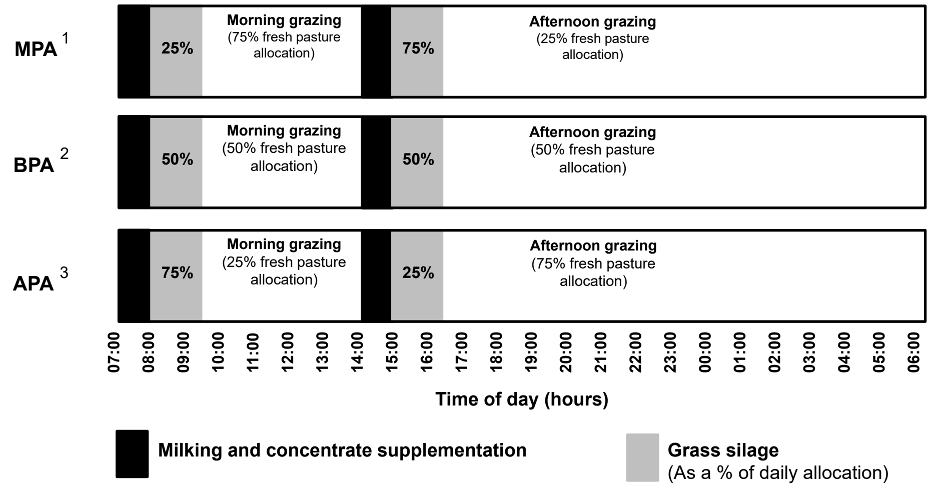

Figure 1. Pasture, grass silage and concentrate allocation of treatments. [1]MPA= 75% of the fresh pasture and 25% of the silage allowance were assigned in the morning; [2]BPA= 50% of the fresh pasture and silage allowance were assigned in the morning; [3]APA= 25% of the fresh pasture and 75% of the silage allowance were assigned in the morning.

Grazing management

The study was run on 12 ha of *Lolium perenne* L. dominated sward (55% *L. perenne*, 33% *Bromus valdivianus*, 5% *Trifolium repens* and 7% of other species), subdivided in 9 paddocks, which were not fertilized during experiment. All treatments were allocated within the same paddock, separated by an electric fence. The cows had access to new fresh pasture allowances at 10:00 h or 16:00 h according to the treatment.

Pre- and post-grazing pasture mass (kg DM/ha, ground level) were estimated three times per week from 100 compressed sward height measurements using a rising plate meter (Ashgrove Plate Meter, Hamilton, New Zealand).

Pasture and supplement sampling and analyses

Pre-grazing pasture samples were collected once a week at 10:00 h (MPA and BPA) and 16:00 h (APA and BPA) before cows had access to the new daily strip, cutting at 4 cm height. Samples of grass silage and concentrate were collected on days 17, 40 and 59 of the experiment. All samples were immediately frozen at -20°C and then freeze-dried for chemical analysis. Before chemical analysis, pasture and supplement samples were ground through a 1-mm screen (Willey Mill, 158 Arthur H, Thomas, Philadelphia, PA, USA), and analyzed for DM, CP, acid detergent fiber (ADF), ash (AOAC, 1996), neutral detergent fiber (NDF) (Van Soest *et al.*, 1991) and metabolizable energy (ME) (Tilley & Terry, 1963; Goering & Van Soest, 1970). Soluble carbohydrates were determined using near-infrared spectroscopy (R^2 of equation = 0.97). D-value was estimated using *in-vitro* digestibility (Tilley & Terry, 1963). The pH and N ammonia in silage samples were derived by the method of AOAC (1996).

Dry matter intake and grazing behavior

Daily DMI was estimated using the indigestible marker technique (Penning, 2004) during days 49 and 62 of the trial. All cows were given paper capsules containing chromium oxide (6 g/d, 68% wt/wt) after each milking, using an oral dispenser. Over the last 6 days, feces samples were collected twice per day from the rectum of each cow at the time of dosing. Fecal concentration of Cr was later determined by atomic absorption spectroscopy (Spectronic Genesys 5 spectrophotometer, Milton Roy, Ivyland, PA, USA). Total and pasture DMI from chromium oxide excretion were calculated as described by Pulido *et al.* (2015).

Individual grazing behavior was recorded over a 24-h period during days 18 and 45 of the experiment. Grazing activities (grazing, ruminating and idling) were observed by three experienced observers (one for each treatment) every 10 min during daylight hours and every 15 min during evening hours.

Milk yield, BW and body condition score

Milk yield was measured daily during each milking with an automated system (MPC580 DeLaval). Milk samples were collected once a week throughout the experiment during a morning and afternoon milking. These samples were used to estimate protein, fat and urea in milk by infrared spectroscopy (Milko-scan, System 4300, Foss Electric, Denmark).

Daily BW was recorded after each milking events with an automated system in the milking parlor. Body condition score (BCS) was evaluated once a week after morning milking by an experienced observer using a 5-point scale (Ferguson *et al.*, 1994).

Ruminal concentration of volatile fatty acids

Ruminal concentration of volatile fatty acids (VFA) were evaluated using the rumen cannulated cows. Individual rumen samples were collected from three locations in the rumen (cranial, ventral and caudal) at 08:00, 10:00, 13:00, 16:00, 20:00, 00:00 and 03:00 hours during days 14, 28 and 42 of the experiment. Immediately after collection of rumen fluid, the samples (from each ruminal site) were bulked and frozen for chemical analysis. Samples were thawed and a subsample of 4 mL was diluted with formic acid (4:1 ratio) and rumen concentration of VFA was determined by gas chromatography (Shimadzu GC-2010 plus High-end GC, equipped with GC Capillary Column, SGE, BP21) (Bal *et al.*, 2000).

Nitrogen partitioning

N partitioning was carried out between weeks 7 and 8 of the experiment. The same bulked fecal samples used to estimate pasture DMI were used to quantify N concentration by a N autoanalyzer LECO FP528 based on DUMAS method (AOAC, 1996). N partitioning was calculated using the equations proposed by Whelan *et al.* (2012):

N intake = [(pasture DMI × % N in pasture ÷ 100) + (concentrate DMI × % N in concentrate ÷ 100) + (grass silage DMI × % N in grass silage ÷ 100)];

Fecal N = (fecal DM excretion × % N in feces ÷ 100), where fecal DM excretion = Total DMI (1−dietary DM digestibility) (Burke *et al.*, 2008);

Milk N = (milk yield × % N in milk ÷ 100);

Urine N = (N intake – fecal N – milk N).

Statistical analysis

Chemical composition of pasture and pasture mass (pre- and post-grazing) were analyzed using a mixed model procedure (PROC MIXED; SAS, v9.4). The model included the fixed effects of treatment, day of sampling, and their interaction, and the random effect of paddock. As day of sampling and interaction were not significant, they were dropped from the model.

Dry matter intake, N partitioning, change in BW and change in BCS were analyzed using a mixed model procedure (PROC MIXED; SAS, v9.4). The model included the fixed effects of treatment and the random effect of cows.

Milk production, milk composition, grazing behavior and rumen parameters were analyzed as repeated measures in time using the mixed model procedure (PROC MIXED; SAS, v9.4). Statistical model included the fixed effects of treatment, random effect of cows, time of sampling as repeated measurement and interaction between treatment and day of sampling. Milk production and body condition score were analyzed including the pre-experimental period as a covariate.

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 pasture

Results of chemical composition of pasture are presented in Table 1. Dry matter content was greater for afternoon pasture than morning pasture ($p=0.03$) and WSC and WSC/CP ratio tended to be greater in the afternoon pasture ($p=0.07$ and $p=0.09$, respectively). Other parameters of chemical composition of pasture were not modified by treatments ($p>0.05$).

Dry matter intake and grazing behavior

Results of DMI and grazing behavior are presented in Table 2. Treatments did not modify total and pasture DMI, which averaged 15.6 and 9.1 kg DM/cow, respectively ($p>0.05$). However, pasture WSC intake tended to be greater for APA than other treatments ($p=0.1$). Grazing time between morning and afternoon milking was longer for MPA ($p<0.01$), while grazing time between afternoon and morning milking was longer for APA compared to other treatments ($p<0.01$). Treatments did not modify total grazing, ruminating and idling time ($p>0.05$).

Milk production and change in BW and BCS

Results of milk production and change in BW and BCS are presented in Table 3. Milk production was unaffected by treatment ($p=0.99$), averaging 22.8 kg/d. Fat, protein and urea in milk were similar between treatments ($p>0.05$) (4.4%, 3.2% and 6.7 mmol/L, respectively).

Table 1. Chemical composition of morning and afternoon pasture and supplements offered to dairy cows under autumn grazing conditions.

Item[1]	Herbage pasture			*p*-value	Supplements			
	Morning	Afternoon	SEM[2]		Grass silage	SEM[2]	Concentrate	SEM[2]
DM	10.6b	11.9a	0.22	0.03	52.4	2.4	87.7	0.08
CP	24.2	23.5	0.42	0.44	14.8	0.5	14.7	0.41
NDF	48.5	50.0	0.93	0.45	52.9	1.2	18.9	1.39
ADF	25.7	25.0	0.29	0.32	29.4	0.9	8.8	0.66
ME	11.13	11.17	0.17	0.86	11.37	0.09	12.13	0.17
Ash	10.8	10.4	0.35	0.53	9.5	0.5	9.3	0.55
WSC	6.12	7.80	3.72	0.07	-	-	-	-
WSC/CP	0.25	0.33	0.02	0.09	-	-	-	-
VD %	73.2	73.6	1.25	0.70	74.9	0.66	80.6	1.28
pH	-	-	-	-	5.54	0.1	-	-
$N\text{-}NH_3$	-	-	-	-	5.21	0.7	-	-

[1] DM, dry matter (% DM); CP, crude protein (% DM); NDF, neutral detergent fiber (% DM); ADF, acid detergent fiber (% DM); ME, metabolizable energy (MJ ME kg/DM); WSC, water soluble carbohydrates (% DM); VD: digestibility value; $N\text{-}NH_3$, ammonia-N (% DM). [2] Standard error of the mean. Means within a row with different letter differ ($p<0.05$).

Table 2. Pasture mass, dry matter intake and grazing behavior of grazing dairy cows under autumn conditions.

Item	Herbage pasture			SEM[2]	*p*-value
	MPA	BPA	APA		
Pasture mass, above ground level					
Pre-grazing, kg DM/ha	3010	2946	3010	60.1	0.89
Post-grazing, kg DM/ha	1656	1699	1690	19.6	0.65
Dry matter intake, kg DM/cow					
Pasture	9.2	8.9	9.1	0.7	0.92
Grass silage	3.0	3.0	3.0	-	-
Concentrate	3.5	3.5	3.5	-	-
Total	15.7	15.4	15.6	0.7	0.92
Herbage WSC[3]	0.57	0.61	0.71	0.05	0.10
Neutral detergent fiber	6.73	6.60	6.80	0.33	0.91
Metabolizable energy[4]	181	176	179	7.53	0.91
Grazing time					
Total, min	357	340	332	6.1	0.24
07:00 - 15:50 h, min	169a	133b	89c	5.9	<0.01
16:00 - 06.45 h, min	188c	207b	243a	5.6	<0.01
Rumination time, min	340	343	349	6.6	0.84
Total idling time, min	677	689	683	8.8	0.92

[1] MPA= 75% of the fresh pasture and 25% of the silage allowance were assigned in the morning; BPA= 50% of the fresh pasture and silage allowance were assigned in the morning; APA= 25% of the fresh pasture and 75% of the silage allowance were assigned in the morning. [2] Standard error of the mean. [3] Water soluble carbohydrates. [4] Metabolizable energy intake = MJ ME/animal/d. Means within a row with different letter differ ($p<0.05$)

Table 3. Milk production, milk solids and change in body weight and body condition score of grazing dairy cows under autumn conditions.

Item	Treatment[1]			SEM[2]	*p*-value
	MPA	BPA	APA		
Milk production, kg/d	22.7	22.7	22.8	1.2	0.99
Milk solids, kg/d	1.72	1.68	1.72	0.09	0.94
Fat, %	4.39	4.28	4.44	0.18	0.82
Protein, %	3.22	3.10	3.28	0.07	0.19
Urea, mmol/L	6.84	6.96	6.40	0.19	0.10
Change in BW[3]	-0.08	-0.14	-0.11	0.02	0.49
Change in BCS[4]	-0.02	-0.01	0.00	0.01	0.32

[1] MPA= 75% of the fresh pasture and 25% of the silage allowance were assigned in the morning; BPA= 50% of the fresh pasture and silage allowance were assigned in the morning; APA= 25% of the fresh pasture and 75% of the silage allowance were assigned in the morning. [2] Standard error of the mean. [3] Body weight. [4] Body condition score. Means within a row with different letter differ ($p<0.05$)

Ruminal concentration of VFA

Results of rumen fluid concentrations of VFA are presented in Table 4 and Figure 2. Propionate molar proportion was greater ($p=0.03$) for BPA than APA, but similar between BPA and MPA. No differences were observed for acetate, butyrate and concentration of total VFA ($p>0.05$). It was not observed interaction between treatment and time of the day (hour) ($p>0.05$).

Nitrogen partitioning

Results of N partitioning are presented in Table 4. Treatments did not modify N intake and its partitioning into milk, urine and feces ($p>0.05$), averaging 458, 119, 195 and 144g N/d. Treatments did not modify N use efficiency (milk N/N intake), which ranged between 25-27% ($p=0.09$).

Discussion

Times of pasture allocation (Abrahamse *et al.*, 2009; Pulido *et al.*, 2015; Vibart *et al.*, 2017) has been evaluated separately with positive (Pulido *et al.*, 2015) or no effects (Abrahamse *et al.*, 2009; Vibart *et al.*, 2017) on milk production, with no effect on N partitioning (Vibart *et al.*, 2017) in grazing dairy cows. On the other hand, time of silage allocation have had a positive (Al-Marashdeh *et al.*,

Table 4. Parameters of rumen fermentation and N partitioning of grazing dairy cows under autumn conditions.

Item	Treatment[1]			SEM[2]	*p*-value	
	MPA	BPA	APA		Treat.	Treat. × Time
Volatile fatty acid (mmol/L)	79.4	76.4	72	4.17	0.46	0.84
Acetate (mol/100 mol)	55.9	55.2	55.5	0.89	0.84	0.69
Propionate (mol/100 mol)	18.6a	18.8a	17.7b	0.41	0.03	0.34
Butyrate (mol/100 mol)	11.2	10.9	11.5	0.31	0.21	0.65
A:P ratio[3]	3.05	2.94	3.16	0.09	0.17	0.39
N partitioning (g/d)						
N intake (g/d)	461	450	464	13.2	0.91	-
Milk N (g/d)	124	113	121	3.4	0.39	-
Fecal N excretion (g/d)	143	145	145	1.4	0.82	-
Urinary N excretion (g/d)	193	193	198	10.9	0.98	-
Milk N/N intake (%)	27	25	26	0.3	0.09	-

[1] MPA= 75% of the fresh pasture and 25% of the silage allowance were assigned in the morning; BPA= 50% of the fresh pasture and silage allowance were assigned in the morning; APA= 25% of the fresh pasture and 75% of the silage allowance were assigned in the morning. [2] Standard error of the mean. [3] Body weight. [4] Body condition score. Means within a row with different letter differ ($p<0.05$)

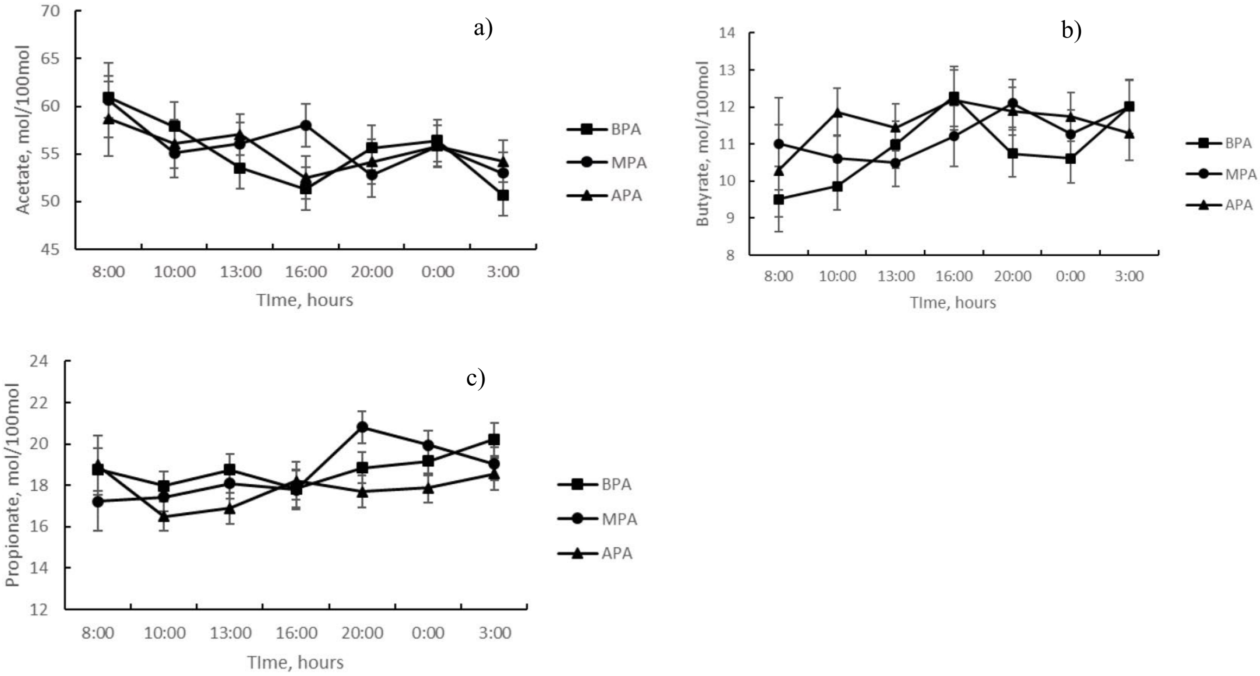

Figure 2. Effect of time of pasture and grass silage allocation of ruminal concentration of a) acetate, b) butyrate and c) propionate of grazing dairy cows. MPA= 75% of the fresh pasture and 25% of the silage allowance were assigned in the morning; BPA= 50% of the fresh pasture and silage allowance were assigned in the morning; APA= 25% of the fresh pasture and 75% of the silage allowance were assigned in the morning.

2016b; Ueda *et al.*, 2017) or non-effects (Al-Marashdeh *et al.*, 2015, 2016a; Mattiauda *et al.*, 2019) on milk production. Similarly, time of silage allocation had a positive (Al-Marashdeh *et al.*, 2015) or non-effects (Al-Marashdeh *et al.*, 2016a,b) on N partitioning, suggesting that profitability goes against the environmental goals (reduction in the urinary N excretion goals, similar than reported by Beltran *et al.* (2019a).

All these studies have evaluated the timing of energy supplements, but there is a lack of information on the literature about the effect of time of grass silage allocation, the most common supplement offered to autumn calving cows during autumn-winter where pasture is limited (Ruiz-Albarran *et al.*, 2016). Therefore, there are no studies evaluating the combination of time of fresh pasture and grass silage allocation as nutritional strategies at the same time.

Chemical composition of pasture

Afternoon pasture showed a greater DM content and tended to have greater WSC content than morning pasture, which could be related to the loss of surface moisture and accumulation of simple sugars from the process of photosynthesis in the plant (Delagarde *et al.*, 2000). The tendency to greater WSC content in the afternoon pasture could be explained by the lower day-length for autumn compared to spring (Delagarde *et al.*, 2000), suggesting that the degree of sugar accumulation in the afternoon may vary depending on the season of the year. Similar results were found in other experiments evaluating time of pasture allocation (Pulido *et al.*, 2015; Vibart *et al.*, 2017).

Grazing behavior and DMI

The time that cows spent grazing after morning and afternoon milking were longer depending on when cows received most of their fresh pasture allowance – cows receiving 75% of fresh pasture in the morning (MPA) grazed for longer in the morning and less in the afternoon than the cows receiving 75% of their fresh pasture allowance in the afternoon (APA), and vice versa. These results were expected, considering that cows tend to spend more time grazing when a new fresh pasture is allocated (Vibart *et al.*, 2017). In this sense, time of pasture allocation allowed for a change in the intensity of the most important grazing bouts throughout the day (dawn and dusk bouts) (Gregorini, 2012), independently of time of grass silage allocation. Despite the lack of effect of time of grass silage allocation on intake and milk production, grass silage should be used during autumn season to maintain a high DMI and thereby, satisfy the milk production requirements of autumn-calving cows (Morales *et al.*, 2014; Ruiz-Albarran *et al.*, 2016).

Despite these changes in grazing intensity in the most important grazing bout, total grazing time and pasture DMI were similar among treatments. A greater DMI for

APA than other treatments was expected; however, pasture and total DMI were similar among treatments, suggesting that shorter grazing time in the morning and longer grazing time in the afternoon for APA were not enough to increase pasture DMI. This could be associated to the lower daylight hours available for grazing in autumn compared to spring (Pulido *et al.*, 2015), which can limit the afternoon grazing time for APA, minimizing any effects of time of silage allocation on pasture intake. Similar results were reported by Sheahan *et al.* (2013), where morning or afternoon allocation of energy supplement did not modify pasture intake.

The cows in all treatments spent more time grazing after afternoon milking. This agreement between treatments occurs in response to diurnal grazing patterns in ruminants, with a daily frequency of three to five grazing bouts with the dusk grazing being the longest and most intense (Gregorini, 2012). The longer grazing time in the afternoon is supported by Gregorini (2012), who hypothesized that ruminant animals aim to maximize rumen fill before the time when predation risk and vigilance increase (night), allowing a stable release of nutrients during this time. Similar results have been reported in other experiments evaluating the time of pasture allocation in grazing dairy cows (Abrahamse *et al.*, 2009; Pulido *et al.*, 2015; Vibart *et al.*, 2017).

Milk production and composition

A greater milk production was expected for cows receiving 75% of their fresh pasture allowance in the afternoon (APA), since longer and most intense grazing events occur at this time (Gregorini, 2012), as pasture has higher WSC/CP ratio compared to morning pasture (Gregorini, 2012; Vibart *et al.*, 2017). However, in our experiment milk production was not affected by treatments, which can be associated with the similar DMI among treatments. Therefore, the tendency for greater WSC intake for APA was not enough to improve the energy intake, which is the main nutrient limiting milk production.

Protein in milk was not affected by treatments, in response to similar energy supply (Pérez-Prieto & Delagarde, 2012), while similar milk fat between treatments can be associated with the similar rumen acetate:propionate ratios (Abrahamse *et al.*, 2009). In contrast with our findings, Pulido *et al.* (2015) reported an increased milk protein concentration for cows allocated to a pasture strip in the afternoon.

Rumen fermentation

Rumen fluid concentration of propionate was greater for MPA than APA, indicating a change in the diurnal energy flow among treatments, which is supported by the numerically greater propionate concentration by MPA compared to APA in the evening-night (see Fig. 2). In this way, it is possible that morning grass silage allocation (APA) after a longer fasting period did not improve ruminal propionate as a consequence for low WSC content from grass silage (Ueda *et al.*, 2016), while morning allocation of pasture improve the ruminal ratio of WSC:CP at the time of 75% of pasture allocation in the afternoon, supporting the numerical peak at 20:00 h. This is supported by Beltran *et al.* (2019b), who found that ruminal concentration of N-NH_3 was greater two hours after morning allocation of grass silage for cows receiving a new pasture allocation in the afternoon, suggesting that high protein degradability of grass silage and its low WSC content reduced the N utilization by microorganism. The tendency for greater pasture WSC intake for APA was insufficient to compensate the energy imbalance from morning silage allocation, which is supported by the similar ME intake among treatments. Rumen fluid concentration of acetate was not affected by treatments, in response to similar NDF intake (Abrahamse *et al.*, 2009), which is the main precursor for acetate synthesis.

Nitrogen partitioning

The lack of effect of timing of pasture and silage allocation on N intake can be attributed to insufficient increase of WSC/CP ratio in the afternoon pasture (Bryant *et al.*, 2014). This result suggests that longer afternoon grazing time for APA was unable to modify CP intake in response to similar CP content compared with MPA. Similar results have been reported for autumn (Vibart *et al.*, 2017) and spring (Bryant *et al.*, 2014) conditions, where grazing dairy cows receiving a morning or afternoon pasture allocation had similar N intakes. Moreover, similar N intake between treatments would explain the lack of effect on urinary N excretion, in response to a positive relationship between N intake and its excretion through urine (Pacheco & Waghorn, 2008).

According to Keim & Anrique (2011), an increase in WSC and a reduction in CP concentrations respectively are required to enhance nitrogen use efficiency (NUE), suggesting that a greater WSC/CP in in the afternoon pasture was not enough to improve NUE as a consequence to a similar CP content between morning and afternoon pasture. The mean NUE value observed in this study (27%) was greater than those previously reported (Al-Marashdeh *et al.*, 2016a; Trevaskis *et al.*, 2004), who evaluated the timing of energy supplements such as maize silage or cereal based-concentrate, that it could be associated with the amount of pasture in the diet and the stage of lactation of the cows. In the current study, pasture represented 58% of the diet, a considerably lower amount than the 85% and 77% described by Al-Marashdeh *et al.* (2016a) and Trevaskis *et al.* (2004) respectively, suggesting that the lower proportion of pasture in the diet allowed to reduce N intake increasing the NUE. In addition, the use of cows in early lactation in the current study in contrast with those with cows in middle and late lactation (Trevaskis *et al.*, 2004; Al-Marashdeh *et al.*, 2016a, respectively) indicate that N partitioning into milk protein is greater in early lactation than late lactation, as previously reported (Miller *et al.*, 2001; Moorby *et al.*, 2006).

The lack of effect of time of pasture and grass silage allocation on NUE and urinary N excretion can be related to the similar urea in milk between treatments. Urea in milk have been suggested as a farm tool to estimate the urinary N excretion (Spek *et al.*, 2016) and protein utilization in the gastrointestinal tract of dairy cows (Guliński *et al.*, 2016). When the supply of amino acids in the rumen are in excess and rumen energy is limited, amino acids are deaminated and excreted by ruminal bacteria as NH_3, which is transported into the liver to be converted to urea, then flowing into the milk, recycled into the rumen via saliva, and then excreted via urine (Pacheco & Waghorn, 2008; Beltran *et al.*, 2019a). Similar rumen degradation of protein (87% and 89%, data unpublished) and organic matter (74% ad 78%) between morning and afternoon pasture supports the idea of similar N utilization by ruminal microorganisms, explaining the lack of effect on urea in milk, NUE and urinary N excretion.

More research is needed to evaluate the effect of the time of pasture and supplement allocations on milk production, rumen function and N partitioning, using high WSC and low CP content supplements which could promote bigger changes in grazing behavior and flow of energy and N throughout the day.

In summary, time of fresh pasture and grass silage allocation had a minimal effect on the chemical composition of pasture (DM and WSC content of pasture), grazing time and ruminal fermentation. However, milk production and N excretion were similar among treatments, possibly associated with the similar pasture and nutrient intake among treatments.

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