Milk production and composition, nitrogen utilization, and grazing behavior of late-lactation dairy cows as affected by time of allocation of a fresh strip of pasture

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ABSTRACT

Eighty late-lactation dairy cows were used to examine the effects of allocating a new pasture strip of a sward based on ryegrass (*Lolium perenne* L.) in the morning (a.m.; ~0730 h) or in the afternoon (p.m.; ~1530 h) on milk production and composition, nitrogen (N) utilization, and grazing behavior. Cows grazed the same pasture strips for 24 h and were offered the same daily herbage allowance. Herbage composition differed among treatments; p.m. herbage had greater dry matter (DM; 22.7 vs. 19.9%), organic matter (OM; 89.5 vs. 88.9%), and water-soluble carbohydrate (10.9 vs. 7.6%) concentrations and lesser crude protein (20.5 vs. 22.2%) and neutral detergent fiber (48.8 vs. 50.4%) concentrations compared with a.m. herbage. Total fatty acids (FA), α -linolenic acid, and polyunsaturated FA (PUFA) were greater in a.m. herbage, whereas monounsaturated FA were greater in p.m. herbage. Estimates of herbage DM intake did not differ among treatments. Daily milk yields and milk fat and milk protein concentrations were similar among treatments, whereas milk fat (684 vs. 627 g/cow), milk protein (545 vs. 505 g/cow), and milk solids (milk fat + milk protein) yields (1,228 vs. 1,132 g/cow) tended to be greater for cows on p.m. herbage. Rumenic acid and total PUFA in milk were greater for cows on a.m. herbage, whereas oleic acid was greater for cows on p.m. herbage. Estimates of urinary N excretion (g/d) did not differ among treatments, but urinary N concentrations were greater for cows on a.m. herbage (5.85 vs. 5.36 g/L). Initial herbage mass (HM) available (kg of DM/ha) and instantaneous HM disappearance rates (kg of DM/ha and kg of DM/h) did not differ, but fractional disappearance rates (0.56 vs. 0.74 per hour for a.m. vs. p.m., respectively) differed. Under the current conditions, timing of pasture strip allocation altered the herbage nutrient supply to

cows; allocating a fresh strip of pasture later in the day resulted in moderate increases in milk and milk solids yields in late-lactation dairy cows. Conversely, a greater concentration of precursor FA in a.m. herbage resulted in a greater concentration of beneficial FA in milk, compared with cows on p.m. herbage.

Key words: grazing management, time of herbage allocation, nitrogen utilization, milk fatty acids

INTRODUCTION

In New Zealand's pastoral dairy farming, nutrients supplied to dairy cows are largely derived from grazed herbage (Holmes et al., 2003; Macdonald et al., 2008), particularly from swards based on perennial ryegrass (*Lolium perenne* L.). Previous experiments (Cosgrove et al., 2007; Doreau et al., 2007) and modeling exercises (Ellis et al., 2009) have examined the effect of ryegrass-based diets with greater water-soluble carbohydrate (WSC) concentrations on lactation performance and N utilization. Dietary (i.e., plant) manipulation in these studies was largely via selective breeding (Miller et al., 2001; Taweel et al., 2006) and management-type approaches (Doreau et al., 2007). Among the grazing management strategies explored, diurnal accumulation of photosynthates accounted for consistent contrasts between WSC and CP concentrations in herbage (Mayland et al., 2005; Gregorini et al., 2008; Cosgrove et al., 2009).

The nutritive value of herbage from temperate swards often increases during the day (Burns et al., 2005; Gregorini et al., 2008), which is attributed to the diurnal processes of plant photosynthesis and transpiration. Dry matter and WSC accumulate during the day (Mayland et al., 2005; Gregorini et al., 2008), often diluting fiber and N concentrations of herbage DM (Delagarde et al., 2000) and enhancing digestibility (Abrahamse et al., 2009) and palatability (Fisher et al., 1999). Also, from dawn to dusk, herbage biomechanical properties (i.e., reduced toughness) enhance the feeding value for herbage consumed during dusk (Gregorini et

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al., 2009b). These diurnal changes affect the temporal pattern of herbage intake and alter the ingestive and digestive behavior of grazing ruminants (Gibb et al., 1998; Orr et al., 2001; Gregorini, 2012).

The consumption of a more balanced fermentable carbon to nitrogen (C:N) ratio from herbage can potentially enhance herbage DMI, milk yield, and N utilization in grazing dairy cows, particularly those in late lactation (Miller et al., 2001; Cosgrove et al., 2007; Brito et al., 2008). Increases in milk production have been attributed to greater digestible DMI (Miller et al., 2001; Brito et al., 2008) and total DMI (Brito et al., 2008). These studies, however, included indoor (Miller et al., 2001) and baleage feeding (Brito et al., 2008) rather than grazing (Cosgrove et al., 2007). However, no intake measurements or estimates were reported in the latter study.

Feeding fresh grass is an efficient way of increasing the content of beneficial fatty acids (FA) in milk, such as n-3 FA and rumenic acid (the major conjugated linoleic acid CLA isomer C18:2 *cis*-9,*trans*-11) (Elgersma, 2015). Despite extensive ruminal biohydrogenation, the amount and quality of herbage consumed, and more specifically, the concentration and fate of FA in feed, digestive tract, and mammary gland, determine the FA profile in milk. Although extensive reviews on FA profiles of milk fat (including those originating from rotational grazing systems) have been published (e.g., Glasser et al., 2013; Elgersma, 2015), the effect of time of allocation of a fresh strip of grass on the FA profile of milk remains to be elucidated.

We hypothesized that late-lactation dairy cows with access to a new pasture allocation (sole diet) after the afternoon milking produce more milk and milk solids while having improved overall N utilization. The objective of this study was to examine the effects of allocating a 24-h strip of fresh herbage from a ryegrass-based pasture either in the morning or in the afternoon on milk production and milk composition (with an emphasis on FA concentrations), N utilization, and grazing behavior in late-lactation dairy cows.

MATERIALS AND METHODS

Research Site, Cows, and Pastures

The experiment was conducted at the Massey University No. 4 Dairy farm (40°19'S, 157°37'E; Palmerston North, New Zealand) during April and May 2010. The research site is located on Tokomaru silt loam soils, defined as Aeric Fragiagualfs with poor natural drainage, at an elevation of 60 m above sea level, with an average annual rainfall of approximately 1,000 mm. Weather

data were obtained from a weather station located in the proximity of the research site (AgResearch, Grasslands Research Centre, Palmerston North).

Eighty lactating cows (Holstein Friesian × Jersey crosses) averaging [mean ± standard deviation (SD)] 463 ± 46.8 kg of BW, 224 ± 21 DIM, and 12.2 ± 2.1 kg initial milk yield were used in this experiment. Cows were blocked by milk yield, parity, and DIM and randomly assigned to either a morning (a.m.; 0730 h, following the morning milking) or an afternoon (p.m.; 1530 h, following the afternoon milking) new pasture strip allocation. Corresponding milking times were approximately 0630 h and 1430 h. Each allocation treatment included 2 herds of 20 cows each (40 cows per treatment). At any given time, all herds (i.e., groups of cows) grazed side-by-side within the same pre-assigned paddock; herds were randomly assigned to strips within paddocks (4 strips per day). Seven paddocks, comprising a total area of 14.5 ha, were used throughout the experiment. The experiment included a period of adaptation (14 d) followed by a period of data collection (14 d). Cows were individually weighed (Tru-Test, Auckland, New Zealand) after morning milking, once during the adaptation period, and twice during the data collection period. Procedures performed during this experiment were approved by the Grasslands Animal Ethics Committee (Approval No. 12054).

Herbage Intake

Experimental paddocks were rotationally grazed (strip-grazed) with the aid of temporary electric fences. Cows grazed the same strip for a 24-h period and were offered the same daily DM allowance, with a targeted daily herbage DMI of 16 kg per cow (sole diet; calculated from clippings to a 4-cm height). The area offered daily per herd was calculated based on the daily herbage DMI target (kg/cow), pregrazing herbage mass (HM) and postgrazing HM (preHM and postHM, respectively) according to the following equation:

$$\text{Area offered daily (ha)} = (\text{cows per herd} \times \text{target DMI}) / (\text{preHM} - \text{postHM}). \quad [1]$$

Pre- and postgrazing HM were determined using an electronic rising plate meter (Farmworks Precision Farming Systems, Feilding, New Zealand), according to [HM, kg of DM/ha = (158 × herbage height, cm) + 200]. The values recorded with the plate meter were the mean of approximately 50 readings taken randomly throughout the area allocated for grazing by each herd daily. Estimates of daily herbage intake were obtained

by difference between pre- and postgrazing HM (Macon et al., 2003). This methodology only provides an average estimate of DMI for each treatment, with paddock (i.e., estimates from 7 different paddocks), rather than the individual animal, as the experimental unit, according to the following equation:

$$\text{DMI (kg of DM/cow)} = [(\text{preHM} - \text{postHM}) / \text{cows per herd}] \times \text{area offered (ha)}. \quad [2]$$

Milk Production and Milk Composition

Milk yields were recorded automatically at every milking (Alpro, DeLaval, Tumba, Sweden). Milk samples were collected from each cow during the afternoon and following morning milking event 3 times (once during the adaptation period and twice during the data collection period; d 0, d 16, and d 23) and analyzed for fat, CP, lactose, and MUN concentrations and somatic cell counts. Milk samples were collected from proportional in-line samplers and immediately refrigerated at 4°C after collection until processing in the laboratory (i.e., Cosgrove et al., 2007). Milk fat, CP, and lactose composition were obtained using a FT 6000 Fourier Transform infrared analyzer (TestLink, Hamilton, New Zealand). Milk urea concentration was determined in milk supernatant by a urease-UV procedure, using a Flexor E clinical chemistry analyzer (Vital Scientific, Dieren, the Netherlands) and a commercial diagnostic kit for urea (Roche Diagnostic NZ Ltd., Mt. Wellington, Auckland, New Zealand) according to recommendations from the manufacturer. Daily milk solids yield (**MSY**; fat + protein) was calculated using the concentration of fat and CP and the milk yields measured on the days of sample collection. Nitrogen secreted in milk was estimated as milk CP yield divided by 6.38.

Milk FA analysis was conducted as described by Schwendel et al. (2015). Briefly, the method by Toledo et al. (2002) was modified to account for a smaller milk sample volume (1 mL). Samples were extracted with 2-propanol (2 mL) and hexane (1.5 mL), which contained [1,1,1-¹³C] trioctanoin (Larodan Fine Chemicals, Solna, Sweden) as an internal standard. The hexane layer was then dried under N₂ and the milk fat was dissolved in hexane (1 mL) before the addition of 0.1 M KOH in methanol (2 mL). Next, tubes were sealed and heated for 1 h at 50°C to facilitate the trans-esterification process of glycerides into the corresponding FAME. The resulting mixture was neutralized with 0.1 M HCl. The organic solvent layer containing FAME was diluted 1:1 (vol:vol) with hexane and injected into a GC-MS (Shimadzu GC-17A QP5050A, Shimadzu,

Kyoto, Japan). The FAME were separated on a 60-m SGE BPX 70 column (i.d. 0.25 mm; film thickness 0.25 µm; 60 min run time). Injection port and interface temperatures were maintained at 240°C, and the column temperature profile was similar to Schwendel et al. (2015), with helium used as the carrier gas. Forty-nine FAME were identified via retention time and their characteristic ion responses and quantified relative to the internal standard and their corresponding response obtained from the concurrent analysis of an external standard (Supelco FAME Mix C4-C24; Sigma-Aldrich, St. Louis, MO). Fatty acids are expressed as grams per 100 g of total FA.

Urinary Nitrogen

In concurrence with the last 2 milk sampling periods, urine samples were collected immediately before, during, and after morning and afternoon milking to estimate urinary N excretion. Spot urine samples were collected from all cows; a representative sample of midstream urination was collected by 5 independent observers. To induce urination, a stimulatory vulvar massage was applied to the minority of cows that did not urinate voluntarily. Urine samples were collected in a plastic container and immediately placed in ice until further processing and analyses. Immediately after collection, subsamples were used for creatinine analysis; urinary creatinine concentration was obtained using a spectrophotometric assay, a compensated Jaffe reaction in a Hitachi 902 automatic analyzer (NZ Veterinary Pathology, Palmerston North, New Zealand). Urine samples were acidified (30 mL + 250 µL of 6 M H₂SO₄) and stored frozen (−20°C) until further analysis. Total urinary N concentration was obtained using a combustion method (Carlo Erba Nitrogen Analyzer; Carlo Erba, Cornaredo, Italy).

Urinary N (g/d) was estimated using 2 prediction models:

- (a) From urinary creatinine in spot samples, using a creatinine excretion factor of 21.9 mg/kg (Pacheco et al., 2007), according to the following equation:

$$\text{N in urine (g/d)} = [(21.9 \times \text{BW, kg}) / \text{urinary creatinine, mg/kg}] \times \text{urine N (g/kg)}; \quad [3]$$

- (b) From MUN concentration according to the equation of Jonker et al. (1998):

$$\text{N in urine (g/d)} = 12.54 \times \text{MUN (mg/dL)}. \quad [4]$$

Grazing Behavior

The temporal grazing pattern was recorded by trained observers during two 24-h periods, on d 16 and d 22 of the experiment. Grazing and nongrazing activities were recorded every 5 min, which is within the time range of visual grazing time measurements reported by Bargo et al. (2003). For the purpose of this study, grazing was defined as cows head down, in contact with the sward, and engaged in acquiring herbage into the mouth, whereas nongrazing included all other activities (i.e., searching, ruminating, idling). From these data, total grazing time was calculated by multiplying each grazing activity (i.e., proportion of cows grazing at any given time) by a 5-min interval (Figure 1). Herbage disappearance during these periods was assessed by using a rising plate meter (Farmworks Precision Farming Systems) at set time intervals (immediately before grazing, and every 30 min thereafter up to 4 h after commencement of the main grazing events; at 0730 h for cows on a.m. herbage and at 1530 h for cows on p.m. herbage). Plate meter readings followed the same transect in all recordings. Herbage mass disappearance rate was cal-

culated from HM measurements using the fractional disappearance model of Ørskov and McDonald (1979) adapted to grazed HM disappearance (Gregorini et al., 2009a). Herbage mass disappearance rate was obtained by fitting HM data (y) from each grazed strip into the following equation:

$$y = a - b \times [1 - \exp(-c \times \text{time})], \quad [5]$$

where a = pregrazing, available HM (kg of DM/ha); b = potential HM disappearance (kg of DM/ha); and c = fractional disappearance rate of HM (per hour). Time was set at 0, 60, 120, 180, and 240 min after commencement of the main grazing events. Instantaneous HM disappearance rates (kg of DM/ha per hour) were estimated as the first derivative of the function [$y = -b \times c \times \exp(-c \times \text{time})$] for the same time points mentioned above, as outlined by Gregorini et al. (2009a).

Pasture Chemical Composition

Concurrently with the HM disappearance assessment, herbage samples were collected immediately

Figure 1. Proportion of cows grazing a new strip of pasture (1.0 = all cows grazing; 0.0 = no cows grazing) offered either in the morning (a.m.) or in the afternoon (p.m.). The top and third graphs correspond to period 1, the second and bottom graphs correspond to period 2.

before grazing and every 30 min up to 4 h after commencement of the main grazing events ($n = 8$ time points $\times$ 2 herds $\times$ 2 treatments $\times$ 2 periods = 64 herbage samples). Samples were hand-plucked from about 10 sites on each walking transect to mimic herbage grazed by the cows. Samples were immediately placed in ice, transported to the laboratory and stored frozen (-20°C), and subsequently freeze-dried and ground to pass a 1-mm Wiley mill sieve. Chemical composition was obtained by near infrared reflectance spectroscopy (FeedTECH, Palmerston North, New Zealand). All herbage samples were analyzed for ash, WSC, NDF, ADF, CP, and lipid concentrations, along with OM digestibility and ME (MJ/kg of DM) concentration (Corson et al., 1999). Also, subsamples collected during the first 60 min post allocation ($n = 2$ time points $\times$ 2 herds $\times$ 2 treatments $\times$ 2 periods = 16) were analyzed for soluble CP (NPN + soluble protein), degradable protein, neutral-detergent insoluble protein and acid-detergent insoluble protein by wet chemistry (Dairy One Forage Lab Services, Ithaca, NY).

For herbage FA composition, freeze-dried pasture subsamples collected during the first 60 min post allocation ($n = 16$) were extracted and methylated in a one-step incubation procedure with a mixture of toluene (Analar reagent), methanol and sulfuric acid (2.5% vol/vol sulfuric acid in methanol), according to Sukhija and Palmquist (1988). The samples were analyzed with a Shimadzu gas chromatograph (Shimadzu GC 2010, with a flame ionization detector; Shimadzu, Kyoto, Japan). The FAME were separated on a 100-m fused silica column (i.d. 0.25 mm; film thickness 0.2 μm ; Restek Rtx-2330; Restek Corporation, Bellefonte, PA). Injection port and detector temperatures were maintained at 240 and 265 $^{\circ}\text{C}$, respectively, with hydrogen (50 cm/s) used as the carrier gas. Nineteen FAME were identified via retention time and quantified relative to tri-C11 (triundecanoin) used as an internal standard, and their corresponding response was obtained from the concurrent analysis of an external standard (Supelco FAME Mix C4-C24, Sigma-Aldrich). Fatty acids in pasture samples are also expressed as grams per 100 g of total FA.

Statistical Analysis

Dependent variables (i.e., herbage chemical composition, estimates of herbage DMI, milk production and composition, grazing behavior, urinary N) were analyzed using SAS software (version 9.3; SAS Institute Inc., Cary, NC). Herbage chemical composition, measures of herbage availability, and estimates of herbage DMI were analyzed using a mixed model procedure (PROC MIXED; SAS Institute Inc.). The model in-

cluded the fixed effects of treatment, day of sampling, and their interaction, and the random effect of paddock. Day of sampling and interaction effects were deemed not significant and subsequently dropped from the model. Paddock was considered the experimental unit. The effects of time of day at which herbage sample collection occurred (0730, 1130, 1530, 1930 h) were analyzed using a similar procedure; the model included the fixed effects of time of day, day of sampling, and their interaction, and the random effect of paddock. Similarly, day of sampling and interaction effects were deemed not significant and subsequently dropped from the model; mean comparisons were performed using the Tukey test.

Milk yield and milk composition along with urinary N concentrations were analyzed using a mixed model procedure with repeated measurements over time. The models included fixed (treatment) and random (cow within herd) effects. Grazing time was also analyzed using a mixed model procedure, whereas pasture disappearance data were analyzed using a nonlinear procedure (PROC NLIN; SAS Institute Inc.). The model of Ørskov and McDonald (1979) was used to analyze the pasture disappearance data for each herd, using the Marquardt method to obtain the parameter estimates a , b , and c , as outlined by Gregorini et al. (2009a). Comparison of parameters was performed using a mixed model procedure (PROC MIXED; SAS Institute Inc.) with herd as the experimental unit. Significance and trends were established at $P < 0.05$ and $P < 0.10$, respectively.

RESULTS

Weather Conditions

Compared with long-term weather data for the month of April (mean air temperature 11.8 $^{\circ}\text{C}$, mean precipitation 56.8 mm, mean relative humidity 79.9%; 2001–2010), mean air temperature (at a 2-m height) was similar (12.0 $^{\circ}\text{C}$), precipitation was lesser (33.4 mm), and relative humidity was slightly greater (82.5%) (The National Climate Database, National Institute of Water and Atmospheric Research, Wellington, New Zealand). The mean amount of hours of sunlight and daily solar radiation during the experimental period were similar to those reported for a longer term for the month of April (5.1 h/d and 11.0 MJ/m² vs. 5.2 h/d and 11.4 MJ/m², respectively).

Herbage Chemical Composition

Herbage composition differed among treatments; p.m. herbage had greater DM (22.7 vs. 19.9%; $P = 0.008$), OM (89.5 vs. 88.9%; $P < 0.001$), and WSC

(10.9 vs. 7.6%; $P < 0.001$) concentrations and lesser CP (20.5 vs. 22.2%; $P < 0.001$) and NDF (48.8 vs. 50.4%; $P = 0.02$) concentrations compared with a.m. herbage (Table 1). To a lesser extent, lipid concentration tended to be reduced in p.m. herbage (2.8 vs. 3.1%; $P = 0.10$), whereas OM digestibility and ME concentrations were similar among treatments ($78.0 \pm 0.57\%$ and 11.4 ± 0.08 MJ ME/kg DM, respectively). Except for soluble protein, which was greater ($P = 0.02$) for a.m. herbage, all other CP fractions were similar among treatments (Table 1).

α -Linolenic acid (ALA; C18:3 *cis*-9,12,15) and PUFA were greater ($P < 0.05$) in a.m. herbage, whereas lauric acid (C12:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1 *cis*-9), arachidic acid (C20:0), and MUFA were greater ($P < 0.05$) in p.m. herbage (Table 1). A trend ($P < 0.10$) was also observed for greater myristic acid (C14:0), palmitic acid (PA; C16:0), and total saturated FA in p.m. herbage.

Herbage WSC concentrations increased throughout the day, attaining a maximum value at 1530 h (10.3%; commencement of the main p.m. grazing event), whereas other plant constituents varied numerically rather than statistically (Table 2). It is important to note, however, that the mean values of the diurnal pattern in chemical composition presented in Table 2 were derived from only 4 samples collected at each time point (2 herds $\times$ 2 periods), whereas the treatment comparison (Table 1) was derived from 32 herbage samples (2 herds $\times$ 2 periods $\times$ 8 time points) analyzed per treatment.

Herbage Quantity and Intake, Milk Production, and Milk Composition

Herbage mass offered (i.e., pregrazing HM) and refused (i.e., postgrazing HM) during the entire experiment did not differ among treatments; mean ($\pm$ SE) pre- and postgrazing HM were $2,872 \pm 147$ kg of DM/

Table 1. Chemical composition (% of DM unless noted otherwise) of herbage allotted to cows either in the morning (a.m.) or in the afternoon (p.m.) ($n = 64$ herbage samples, unless specified otherwise)

Chemical composition	Treatment		SE	$P \leq$
	a.m.	p.m.		
DM, %	19.9	22.7	0.70	0.008
OM	88.9	89.5	0.09	<0.001
Water-soluble carbohydrates	7.6	10.9	0.45	<0.001
Starch	0.46	0.45	0.27	0.92
NDF	50.4	48.8	0.45	0.02
ADF	25.7	25.8	0.26	0.93
Lipid	3.1	2.8	0.16	0.10
OM disappearance, % of OM	78.1	77.9	0.57	0.85
ME, MJ/kg of DM	11.4	11.4	0.08	0.90
CP	22.2	20.5	0.25	<0.001
Protein fractions, % of CP ($n = 16$ samples)				
Soluble protein	35.4	30.4	2.04	0.02
Degradable protein	73.6	74.4	2.16	0.50
NDICP ¹	6.3	5.9	0.88	0.33
ADICP ²	2.6	2.1	0.76	0.21
FA ($n = 16$ samples)	2.2	1.8	0.10	0.03
Individual FA, g/100 g of FA				
C12:0	2.39	2.94	0.12	0.006
C14:0	0.88	1.08	0.07	0.06
C16:0	25.25	29.43	1.65	0.09
C16:1	0.50	0.63	0.04	0.04
C18:0	2.79	3.31	0.17	0.05
C18:1 <i>cis</i> -9	2.72	3.50	0.19	0.01
C18:1 <i>cis</i> -11	0.49	0.51	0.03	0.62
C18:2 <i>cis</i> -9, <i>cis</i> -12	10.54	9.73	0.57	0.34
C18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	44.96	38.25	2.22	0.04
C20:0	0.75	0.94	0.05	0.02
C22:0	2.66	2.65	0.28	0.99
C24:0	2.41	2.60	0.24	0.59
SFA, % of total FA	38.6	44.6	2.40	0.06
MUFA, ³ % of total FA	4.3	5.5	0.23	0.002
PUFA, ⁴ % of total FA	57.1	49.9	2.52	0.04

¹NDICP = neutral-detergent insoluble protein.

²ADICP = acid-detergent insoluble protein.

³Sum of MUFA.

⁴Sum of PUFA.

Table 2. Diurnal pattern of the main plant constituents of ryegrass (*Lolium perenne*)-based herbage during daylight hours

Time of day	Chemical composition, % of DM ¹						
	OM	NFC ²	WSC ³	NDF	ADF	CP	Lipid
0730 h	88.6	12.5	6.0 ^a	51.5	26.5	21.5	3.0
1130 h	88.8	13.4	8.2 ^{ab}	50.9	26.3	21.6	3.0
1530 h	88.9	14.8	10.3 ^b	48.9	25.5	21.8	3.4
1930 h	89.3	15.2	10.1 ^b	51.3	27.2	19.8	2.9
SE	0.19	1.22	0.95	1.50	0.79	0.69	0.12

^{a,b}Mean values in the same column with different superscripts differ ($P < 0.05$).

¹n = 4 herbage samples per time point.

²NFC = 100 – (ash + CP + NDF + lipid).

³Water-soluble carbohydrates.

ha ($P = 0.39$) and $1,831 \pm 63$ kg DM/ha ($P = 0.46$), respectively (Table 3). Similarly, area offered daily to cows did not differ among treatments (0.393 ± 0.1 ha; $P = 0.26$). Consequently, estimates of herbage DMI from pre- and postgrazing HM did not differ among treatments (12.8 ± 0.7 kg DM/d) (Table 3). Daily milk yields, percent milk fat, and percent milk CP were similar among treatments, whereas milk fat yields (684 vs. 627 g/cow; $P = 0.05$), milk CP yields (545 vs. 505 g/cow; $P = 0.10$), and MSY (1,228 vs. 1,132 g MSY/cow; $P = 0.08$) tended to be greater for cows on p.m. herbage. Milk urea N concentrations (15.2 ± 0.3 mg/dL) and SCS (3.6 ± 0.1) did not differ among treatments.

Four of the 49 milk FA analyzed differed in their relative contribution to total FA (g/100 g total FA; $P < 0.05$) between cows offered an a.m. or a p.m. allocation of a fresh strip of pasture (Table 4). Oleic acid

(C18:1 *cis*-9) and *cis*-vaccenic acid (*cis*-VA; C18:1 *cis*-11) were greater in milk from cows on p.m. herbage, whereas the branched-chain FA *iso*-hexadecanoic acid (*iso*-C16:0) and rumenic acid (RA; CLA isomer C18:2 *cis*-9, *trans*-11) were greater in milk from cows on a.m. herbage. Total PUFA in milk were greater ($P < 0.05$) from cows on a.m. herbage (7.61 vs. 7.22 g/100 g FA; $P = 0.01$), whereas total saturated FA, odd- and branched-chain fatty acids (OBCFA; both total and those of microbial origin), and MUFA were similar among dietary treatments.

Nitrogen Partition

Estimates of N intake (calculated from herbage DMI and N concentration in herbage) tended to be greater for cows on a.m. herbage ($P = 0.09$), whereas N output

Table 3. Herbage mass (HM) offered and refused, estimates of intake, milk production and milk composition from cows offered a new strip of pasture either in the morning (a.m., allotted at 0730 h) or in the afternoon (p.m., allotted at 1530 h)

Variable ¹	Treatment		SE	$P \leq$
	a.m.	p.m.		
Pregrazing HM, kg of DM/ha	2,856	2,889	147	0.39
Postgrazing HM, kg of DM/ha	1,818	1,844	63	0.46
Grazing area, ha/d	0.40	0.39	0.11	0.26
Intake, ² kg/d	12.7	12.9	0.7	0.75
Milk, kg/d	12.2	13.1	0.3	0.16
Milk fat, %	5.15	5.27	0.06	0.16
Milk fat, g/d	627	684	6	0.05
Milk protein, %	4.15	4.19	0.02	0.20
Milk protein, g/d	505	545	9	0.10
Milk solids, ³ g/d	1,132	1,228	17	0.08
MUN, mg/dL	15.0	15.3	0.3	0.51
SCS ⁴	3.57	3.64	0.08	0.52

¹Estimates of the first 4 variables (pre- and postgrazing HM, grazing area, and DMI) were obtained using paddock as the experimental unit, whereas the remaining variables (milk and milk composition) were obtained using animal as the experimental unit (see text for more detail).

²DM basis. Estimates obtained by difference between pre- and postgrazing HM.

³Milk solids = milk fat + milk protein.

⁴SCS = $\{[\log_{10}(\text{SCC}/1000) - 2]/\log_{10}(2)\} + 3$.

in milk tended to be greater for cows on p.m. herbage ($P = 0.10$) (Table 5). Estimates of urinary N excretion, however, did not differ among treatments (220 ± 6.9 g/d and 194 ± 6.2 g/d obtained from Equations [3]

and [4], respectively). Urinary N concentration (g/L) was greater for cows on a.m. herbage (5.85 vs. 5.36 g/L, $P = 0.04$; Table 5) and was higher in samples collected during the morning (6.18 vs. 5.04 g/L, SE =

Table 4. Fatty acid (FA; g/100 g of FA) composition of milk from cows offered a new strip of pasture either in the morning (a.m., allotted at 0730 h) or in the afternoon (p.m., allotted at 1530 h) and time of milking (afternoon or morning)

FA, g/100 g of FA	Time of allocation (A)			Time of milking (M) ¹			$P \leq$		
	a.m.	p.m.	SED ²	Afternoon	Morning	SED	A	M	A $\times$ M
Even-chain SFA									
C10:0	2.43	2.35	0.135	2.33	2.46	0.058	0.64	0.03	0.10
C12:0	2.96	2.87	0.091	2.88	2.95	0.074	0.66	0.35	0.13
C14:0	10.77	10.59	0.278	10.70	10.67	0.151	0.58	0.83	0.009
C16:0	19.96	19.31	0.569	19.57	19.71	0.282	0.38	0.59	0.23
C18:0	8.76	9.08	0.244	9.22	8.62	0.244	0.18	0.02	0.55
C20:0	0.11	0.11	0.005	0.12	0.10	0.003	0.91	<0.001	0.24
C22:0	0.045	0.044	0.0018	0.050	0.039	0.0017	0.43	<0.001	0.42
C24:0	0.027	0.026	0.0011	0.030	0.023	0.0011	0.11	<0.001	0.45
Odd-chain SFA									
C11:0	0.032	0.027	0.0024	0.029	0.030	0.0015	0.20	0.40	0.93
C13:0	0.068	0.062	0.0030	0.066	0.064	0.0017	0.19	0.23	0.88
C15:0	2.58	2.48	0.031	2.59	2.48	0.026	0.08	<0.001	0.36
C17:0	1.14	1.12	0.026	1.16	1.10	0.016	0.45	<0.001	0.30
C21:0	0.045	0.045	0.0019	0.051	0.040	0.0019	0.70	<0.001	0.37
C23:0	0.039	0.038	0.0018	0.043	0.033	0.0018	0.52	<0.001	0.22
Branched-chain FA									
C14:0 <i>iso</i>	0.099	0.096	0.0016	0.102	0.093	0.0013	0.25	<0.001	0.24
C15:0 <i>iso</i>	0.14	0.14	0.003	0.15	0.12	0.003	0.93	0.001	<0.001
C15:0 <i>anteiso</i>	0.82	0.78	0.027	0.82	0.78	0.013	0.30	<0.001	0.29
C16:0 <i>iso</i>	0.70	0.68	0.008	0.72	0.66	0.008	0.01	<0.001	0.001
C17:0 <i>iso</i>	0.73	0.72	0.009	0.76	0.70	0.009	0.37	<0.001	0.26
C17:0 <i>anteiso</i>	0.64	0.61	0.015	0.64	0.607	0.011	0.23	0.001	0.49
C18:0 <i>iso</i>	0.030	0.028	0.0015	0.031	0.027	0.0006	0.29	<0.001	0.02
MUFA									
C14:1	1.21	1.12	0.041	1.18	1.16	0.034	0.16	0.49	0.06
C16:1 <i>cis</i> -9	4.26	4.21	0.117	4.26	4.21	0.117	0.66	0.68	0.37
C16:1 <i>trans</i> -9	0.654	0.642	0.0191	0.667	0.629	0.0142	0.60	0.01	0.57
C17:1 <i>cis</i> -9	0.74	0.90	0.069	0.69	0.95	0.022	0.14	<0.001	<0.001
C18:1 <i>cis</i> -9	16.69	17.34	0.205	17.11	16.92	0.205	0.002	0.38	0.39
C18:1 <i>trans</i> -9	0.61	0.62	0.017	0.65	0.58	0.010	0.77	<0.001	0.38
C18:1 <i>cis</i> -11	2.15	2.26	0.027	2.29	2.11	0.028	<0.001	<0.001	0.35
C18:1 <i>trans</i> -11	7.14	6.67	0.403	7.30	6.51	0.212	0.35	<0.001	0.49
C19:1	0.35	0.36	0.005	0.37	0.34	0.005	0.11	<0.001	0.21
PUFA									
C18:2 <i>cis</i> -9, <i>cis</i> -12	1.51	1.54	0.060	1.59	1.46	0.022	0.67	<0.001	0.76
C18:2 <i>cis</i> -9, <i>trans</i> -11	3.30	2.87	0.131	3.30	2.88	0.130	0.001	0.001	0.74
C18:2 <i>trans</i> -10, <i>cis</i> -12	0.53	0.49	0.026	0.57	0.45	0.016	0.24	<0.001	0.87
C18:3	1.84	1.94	0.097	1.98	1.80	0.037	0.45	<0.001	0.03
C20:2	0.013	0.012	0.0005	0.014	0.011	0.0004	0.35	<0.001	0.22
C20:3n-3	0.071	0.069	0.0060	0.076	0.064	0.0017	0.81	<0.001	0.83
C20:3n-6	0.023	0.023	0.0008	0.026	0.021	0.0007	0.76	<0.001	0.31
C20:4n-6	0.11	0.10	0.009	0.12	0.09	0.003	0.22	<0.001	0.97
C20:5n-3	0.18	0.17	0.012	0.19	0.16	0.004	0.46	<0.001	0.35
C22:6n-3	0.022	0.020	0.0019	0.023	0.018	0.0009	0.36	<0.001	0.02
Total SFA	58.0	58.1	0.46	57.1	59.1	0.45	0.82	<0.001	0.72
Total OBCFA ³	7.89	7.82	0.072	7.93	7.78	0.072	0.29	0.05	0.51
OBCFA microbial ⁴	7.58	7.53	0.072	7.62	7.49	0.072	0.51	0.07	0.57
Total MUFA	34.4	34.7	0.35	35.0	33.9	0.34	0.30	0.001	0.72
Total PUFA	7.61	7.22	0.148	7.88	6.96	0.148	0.01	<0.001	0.79
Total FA, mg/mL	33.4	39.2	3.51	39.5	33.0	1.43	0.24	<0.001	0.36

¹Afternoon (starting at ~1430 h) or morning (starting at ~0630 h) milking.

²SED = standard error of the difference.

³OBCFA = odd- and branched-chain fatty acids.

⁴OBCFA of microbial origin (sum of *iso* C14:0, C15:0, *iso* C15:0, *anteiso* C15:0, *iso* C16:0, C17:0, *iso* C17:0, *anteiso* C17:0, and C17:1 *cis*-9) (Vlaeminck et al., 2006).

Table 5. Nitrogen (N) intake, N in milk, and N in urine from cows offered a new strip of pasture either in the morning (a.m., allotted at ~0730 h) or in the afternoon (p.m., allotted at ~1530 h)

Variable	Treatment		SE	<i>P</i> ≤
	a.m.	p.m.		
N intake ¹ (NI), g/d	475	426	26.8	0.09
N in milk, g/d	79.2	85.4	1.4	0.10
N in milk (proportion of NI)	0.17	0.20		
Concentration of N in urine, g/L	5.85	5.36	0.24	0.04
N in urine, ² g/d	220.0	219.2	6.9	0.93
N in urine (proportion of NI)	0.46	0.51		
N in urine, ³ g/d	192.1	195.2	6.2	0.60
N in urine (proportion of NI)	0.40	0.46		
Creatinine in urine, mg/L	275	268	5	0.40

¹N intake = (estimated DMI, kg/d × CP, %)/6.25.

²N in urine, calculated according to Equation [3] in text: N in urine, g/d = [(21.9 × BW, kg)/urinary creatinine, mg/kg] × urine N, g/kg.

³N in urine, calculated according to Equation [4] in text: N in urine, g/d = 12.54 × MUN, mg/dL.

0.22 g/L; *P* < 0.01) compared with samples collected in the afternoon (data not shown).

Grazing Behavior

Total time spent grazing was similar among treatments (510 ± 11.6 min; *P* = 0.21; Table 6; Figure 1). Time spent grazing during the first 4 h after a.m. milking was greater for cows on a.m. than for cows on p.m. herbage (196 vs. 110 min; *P* < 0.001), whereas time spent grazing during the 4 h after p.m. milking was similar among treatments. Time spent grazing (cows grazing as a proportion of all cows in the herd) is represented in Figure 1. During the grazing behavior observation periods, initial HM available (kg of DM/ha), potential HM disappearance (kg of DM/ha), and instantaneous HM disappearance rates (kg of DM/ha per hour) did not differ among treatments. Fractional disappearance rates, however, were greater for cows on p.m. herbage (0.74 vs. 0.56 per hour; *P* = 0.04; Table 6).

DISCUSSION

Although strip grazing is a commonly adopted grazing method in pastoral dairying, the role of timing of herbage allocation on milk FA profiles has only received limited attention. Despite similar partitioning of N toward urine, allocating a fresh strip later in the day resulted in moderate increases in N captured in milk and milk solids yields in late-lactation dairy cows. Conversely, a greater concentration of beneficial FA in a.m. herbage resulted in a greater concentration of RA and PUFA in milk from cows on a.m. herbage compared with cows on p.m. herbage.

Time of Feed Allocation and Herbage Chemical Composition

In our experiment, DM concentration increased by 14% by offering p.m. versus a.m. forage. Similar differences have been reported for vegetative ryegrass (up to 25%) sampled at 1000 and 1600 h (Pulido et al., 2015) and for vegetative wheat (20%) sampled at either 0800 or 1900 h (Gregorini et al., 2008). Differences in diurnal OM concentration, however, were smaller than those reported by Gregorini et al. (2008) for similar time points (0.8% vs. 6%, respectively); however, initial OM values in the present study were greater than those reported in that study (88.6 vs. 83.0%).

Greater concentrations of WSC are a direct consequence of increased accumulation of photosynthates in leaves as the day progresses; WSC concentration increased by 43% in p.m. herbage compared with a.m. herbage. Similar differences (46%) were reported for ryegrass in autumn by Pulido et al. (2015). Although not statistically significant, NDF concentration throughout the day was greatest at 0730 h, reaching the lowest value at 1530 h and increasing again at 1930 h, similar to the initial NDF concentration measured, a diurnal pattern similar to vegetative wheat (Gregorini et al., 2008) and alfalfa (Burns et al., 2005). An inverse relationship occurred between NDF and both WSC and neutral detergent solubles (100 – NDF), and this pattern was presumably attributed to greater respiration and metabolic activity of plants during the late evening hours. The variations in chemical composition reported in the present experiment are also in agreement with those reported by Delagarde et al. (2000) for samples of perennial ryegrass collected at 0800 and 1900 h.

Increases in WSC concentration at the expense of CP have been attributed to decreased amount of carbon

chains partitioned toward protein synthesis and corresponding decreased energy required in nitrate reduction before protein synthesis (Peyraud and Astigarraga, 1998). Genetic improvements in WSC concentration (i.e., WSC-enhanced cultivars or high-sugar ryegrasses) often, but not always, occur at the expense of structural carbohydrates (i.e., NDF), suggesting an improved carbon partition between carbohydrate fractions for WSC-enhanced cultivars (Miller et al., 2001). Greater concentrations of WSC from a high-sugar perennial ryegrass grown in New Zealand were offset largely by reductions in protein, and to a lesser extent, by reductions in NDF concentration (Cosgrove et al., 2009). Provided these relationships are sustained, concerted efforts toward increasing WSC could lead to further nutritional and environmental benefits from reducing both CP and NDF concentrations from incremental WSC. Overall, results reported herein sustain this premise; the herbage allocated to cows after the afternoon milking was of greater nutritive value to potentially support greater milk production than that allocated after the morning milking.

In addition to perennial ryegrass, exploiting diurnal variation in WSC concentrations as a means of nutritive value enhancement has been reported for other forages such as Italian ryegrass (*Lolium multiflorum* Lam.) (Trevaskis et al., 2004), phalaris (*Phalaris aquatica* L.) (Ciavarella et al., 2000), tall fescue [*Lolium*

arundinaceum (Schreb.) Darbysh.] (Fisher et al., 1999), cocksfoot (*Dactylis glomerata* L.) (Griggs et al., 2005), wheat (*Triticum aestivum* L.) (Gregorini et al., 2008), and alfalfa (*Medicago sativa* L.) (Brito et al., 2008). The photosynthetic processes (and associated sampling time during the main grazing events) had a considerable effect on various plant chemical components. These changes particularly occur in the upper layers of the sward, associated with processes of intense photosynthesis and gas exchange (Delagarde et al., 2000).

Ruminants offered fresh temperate forages have access to diets rich in PUFA, particularly ALA. A greater concentration of total FA, ALA, and PUFA in a.m. herbage is consistent with values reported by Doreau et al. (2007). Perennial ryegrasses manipulated for divergent soluble carbohydrate (SC):N ratios (+SC/−N vs. −SC/+N) offered as sole diets showed that the −SC/+N ryegrass contained more FA (2.5 vs. 1.8% of DM), a greater proportion of ALA (66 vs. 61% of total FA), and a greater proportion of PUFA (78 vs. 74% of total FA), often at the expense of saturated FA of 14 to 24 C (Doreau et al., 2007). The greater concentrations of FA and ALA in −SC/+N ryegrass were attributed to the association between FA and N in chloroplasts (Doreau et al., 2007), confirming the positive and overall linear relationship between ALA and N concentration in herbage (Elgersma, 2015), also seen in our study. The relationship between FA and

Table 6. Time spent grazing (min) and dynamics of herbage mass (HM) disappearance during the first 4 h of the main grazing event of cows offered a new strip of pasture either in the morning (a.m., allotted at ~0730 h) or in the afternoon (p.m., allotted at ~1530 h)

Variable	Treatment		SE	<i>P</i> ≤
	a.m.	p.m.		
Grazing time, min/d				
Total	525	495	11.6	0.21
First 4 h				
Post-a.m. ¹	196	110	9.9	<0.001
Post-p.m. ²	189	194	8.0	0.67
Parameter ³				
<i>a</i>	3,352	3,256	84.6	0.43
<i>b</i>	1,069	970	59.2	0.24
<i>c</i>	0.56	0.74	0.08	0.04
HM disappearance rate ⁴				
0 min	589	694	69.1	0.33
60 min	324	333	26.6	0.83
120 min	184	163	20.5	0.50
180 min	108	82	17.7	0.33
240 min	65	42	14.3	0.29

¹After morning milking.

²After afternoon milking.

³HM disappearance parameters were obtained by fitting HM data (*y*) from each herd into the equation $y = a - b \times [1 - \exp(-c \times \text{time})]$ (Ørskov and McDonald, 1979), where *a* = pregrazing, available HM, kg of DM/ha; *b* = potential HM disappearance, kg of DM/ha; *c* = fractional disappearance rate of HM, per hour.

⁴HM instantaneous disappearance rates (kg of DM/ha per hour), estimated at 0, 60, 120, 180, and 240 min after commencement of the main grazing events. Calculated from the first derivative of the model above; instantaneous disappearance $y = -b \times c \times \exp(-c \times \text{time})$ (Gregorini et al., 2009a).

SC, however, remains unclear, with no discernible trend (Miller et al., 2001; Scollan et al., 2003). Also, in the current study, the proportions of ALA, PA, and linoleic acid (C18:2 *cis*-9,12) in herbage were consistent with values reported previously for ryegrass-based herbage (Bauchart et al., 1984; Palladino et al., 2009).

Time of Feed Allocation and Intake, Milk Production, and Milk Composition

Daily herbage intake was similar among treatments; these findings agree with those reported for early-lactating (Pulido et al., 2015) and mid-lactating dairy cows (Orr et al., 2001; Abrahamse et al., 2009) grazing a perennial ryegrass-based pasture either in the morning or the afternoon. These authors found no difference in daily herbage DMI in morning versus afternoon allocation of a new strip of pasture. In the present study, although estimates of herbage DMI were similar among treatments, daily milk, milk fat, and milk protein yields increased by 7% (+0.9 kg/d), 9% (+57 g/d), and 8% (+40 g/d), respectively, by offering p.m. versus a.m. forage. Consistent with our findings, Orr et al. (2001) reported a 5% increase in milk yields for cows moved to a new strip of pasture after afternoon milking. Gibb (2006) reported milk fat and milk protein concentrations from the experiment by Orr et al. (2001). Corresponding concentrations were increased by 4.7 and 0.4 g/kg, respectively, from cows on p.m., in broad agreement with our findings (1.2 and 0.4 g/kg, respectively). Although milk yields were similar, Abrahamse et al. (2009) reported increased fat- and protein-corrected milk yields from cows on p.m., generally attributable to greater milk fat concentrations.

Improving the nutritional quality of milk FA is becoming an increasing feature demanded by consumers, and enhancing the concentration of FA that are beneficial for human health could contribute to restore consumers' confidence in milk. Important targets remain reducing the amounts of medium-chain saturated FA and enhancing the contribution of MUFA (i.e., oleic acid and VA) and PUFA (RA and ALA) with known health benefits (e.g., Hebeisen et al., 1993; Hu et al., 1997). The quantity and quality of milk is related to the amount and quality of ingested herbage. In the present study, changing the time of feed allocation (and consequential herbage FA profile offered) altered the FA profile of milk (Table 4). Although a relatively small number of milk FA were altered by means of allocation time, the greater contribution of RA and total PUFA in milk of cows on a.m. herbage is significant and most likely attributable to a greater contribution of C18 precursors in a.m. herbage (largely ALA; Table 1). Rumen microorganisms and postingestive desaturation

activity metabolize most dietary PUFA via complex processes. The concentration of PUFA in milk from grazing cows can be enhanced by increasing the amount or concentration of C18 precursors in the diet, by reducing the extent of ruminal biohydrogenation, or by increasing the mammary gland enzymatic activity of the Δ -9 desaturase complex (i.e., the desaturation of VA into RA). Enhancing the amount or concentration of dietary precursors is most likely the contributing factor to greater RA and PUFA in milk from cows on a.m. herbage.

The strong linear relationship between the overall concentrations of RA and VA in milk [$\text{RA (g/100 FA)} = 0.42 \times \text{VA (g/100 FA)} + 0.18$; $R^2 = 0.47$], consistent with the precursor-product relationship, with VA/RA within 2 to 2.5 (Elgersma et al., 2006) (2.2 and 2.3 for milk from a.m. and p.m. herbage, respectively), were also seen in our study. Also, similar OBCFA concentrations among allocation treatments are in agreement with similar duodenal flows of OBCFA offered perennial ryegrasses manipulated for divergent SC:N ratios (+SC/-N vs. -SC/+N) offered as sole diets (Doreau et al., 2007).

The greater concentrations of RA, total MUFA and PUFA (largely at the expense of saturated FA), and total FA (mg/mL) were realized in milk collected following the a.m. grazing period (i.e., afternoon milking) compared with milk collected following the p.m. allocation period (i.e., morning milking). In overall agreement with our findings, a recent comparison across conventional and organic grazing systems showed greater concentrations of VA, RA, and OBCFA and lesser concentrations of saturated FA up to 16 C in milk collected during the afternoon compared with milk collected during the morning milking (Schwendel et al., 2015). In addition to changes in the FA profile of ingested herbage and grazing behavior (varying herbage disappearance rates in particular, as discussed below), changes in the FA profile of milk may also be attributable to the time since previous milking. In the current experiment, 8 and 16 h elapsed between morning and afternoon milking, respectively, but the effect of different milking intervals on the FA profile of milk remains largely unknown.

Several grazing management strategies including differences in HM offered (Stanton et al., 1997), herbage allowance (Bargo et al., 2006), time spent at pasture (Khanal et al., 2008), harvesting date and harvesting interval (Dewhurst et al., 2001), and N application rates (Elgersma et al., 2005) have been explored. But less is known about the effects of timing of fresh forage allocation (and corresponding milking time) on the FA profiles in milk. Pasture management plays an important role in forage FA composition, and quantifying the

composition of FA in herbage in response to grazing management strategies can aid in the design of grazing strategies to increase precursors of beneficial FA in dairy products.

Time of Feed Allocation and N Partition

Consistent with our findings, Abrahamse et al. (2009) reported similar MUN concentrations among allocation treatments. Changes in milk and blood urea N concentrations have been associated with ammonia N concentration in the rumen (Jonker et al., 1998). Further, changes in ruminal ammonia N concentrations were quickly observable in MUN (Gustafsson and Palmquist, 1993), suggesting that an improved balance in the supply of energy from nonstructural carbohydrates and N leading to reduced ruminal ammonia N concentrations influences excretion of urea N in milk. However, the improved WSC to CP ratio provided by the p.m. herbage may not have been sufficient to elicit a significant response in N excretion, as was evidenced by similar MUN concentration and estimates of urinary N losses (g/d). Nonetheless, the amount of N secreted in milk tended to be greater for cows on p.m., and N utilization efficiency (N in milk expressed as a proportion of N intake) increased by 18% by offering p.m. versus a.m. herbage. Together, these findings suggest an overall improvement in N utilization from cows on p.m. herbage.

Although timing of pasture allocation resulted in similar estimates of N partitioning toward urine (g of N/d), N concentrations in urine (g of N/L) were greater for cows on a.m. herbage. The reduced urinary N concentrations from cows on p.m. herbage is most likely to be attributable to differences in N and WSC concentrations in the herbage. In a multivariate context, Pacheco et al. (2010) reported that the urinary N concentration was inversely related to the DCAD (mEq/kg of DM) and the WSC concentration of the herbage consumed. Also, despite a significant reduction in urinary N concentration in one of the forages tested (a tetraploid Italian ryegrass *L. multiflorum*) compared with 2 diploid ryegrasses, the total amount of N excreted (g/d) did not differ among grasses (Pacheco et al., 2010). In our study, although dietary cation–anion difference, a measure of the difference between cations (Na and K) and anions (S and Cl) in herbage, did not differ, the WSC concentration in herbage was lesser for a.m. herbage. Dilution or concentration of N in urine by increasing or decreasing urine volumes and the number of urination events could also be partly responsible, but urine volumes and number of urination events were not measured in our study. Last, urinary creatinine and

MUN concentrations did not differ among allocation treatments (Equations [3] and [4], respectively).

In addition to total N excretion, high concentrations of urinary N in the urine patch in soil contributes substantially to the environmental impact of pastoral dairying. Further, manipulation of urinary N concentration (g/L) has been proposed as a means for reducing livestock greenhouse gas emissions from dairying in New Zealand (Ledgard et al., 2007). In the present study, both the effects of time of herbage allocation and time of sampling had a considerable effect on urinary N concentration and are important aspects to consider in the design of grazing strategies that target sustainable livestock emissions. Urinary N concentrations presented herein are in agreement with those reported by Pacheco et al. (2010) for dairy cows grazing ryegrass-based pastures in autumn.

Time of Feed Allocation and Grazing Behavior

With dairy cows grazing temperate pastures, the longest and most intense grazing events occur at dusk, and these events are often associated with a greater intake rate at this time of the day (Orr et al., 2001). In addition, it has been widely reported that most of the daily consumption of herbage by nonsupplemented grazing dairy cows occurs during daylight hours and that the proportion of time spent grazing is often greatest following the allocation of cows to a fresh strip of pasture (Abrahamse et al., 2009; Gregorini et al., 2009a). In the present study, total grazing time by cows on the a.m. treatment increased by 6% (+30 min), with more of the grazing activity occurring during daylight hours (84 vs. 72% of total grazing time). However, cows on a.m. herbage spent more time grazing during the first 4 h after morning milking, but the time spent grazing after afternoon milking remained similar among treatments. Although estimates of daily herbage DMI were similar among a.m. and p.m. cows, the above findings, along with a different herbage disappearance rate (per hour), suggest that the temporal pattern of intake differed among allocation treatments. Cows given a new pasture strip in the afternoon obtained more of their herbage DMI in the evening, when herbage WSC concentrations were greater and CP concentrations were lesser (Table 2).

In similar experimental setups, Orr et al. (2001) and Abrahamse et al. (2009) reported a similar intake pattern for dairy cows allocated to either a.m. or p.m. herbage. These authors reported that cows allocated to a.m. herbage tended to have lower bite masses in the afternoon, but greater effects (even lower bite masses) were observed for cows on p.m. grazing in the morning.

These findings are consistent both with an effect of sward depletion as the hours of grazing progressed beyond the main grazing event and with the greater rate of HM disappearance by cows allotted to fresh p.m. herbage. Because the quantity of herbage DM a grazing animal can prehend at any given bite represents a small fraction of the total daily requirement, reductions in bite size caused by adverse sward conditions must be compensated if daily intake is to be maintained. Attempts to compensate for reductions in bite size are often performed via increased grazing time (i.e., cows on a.m.) or increased biting rate (i.e., cows on p.m.). Assuming that more than 70% of the daily intake of herbage from pasture occurs within the first 3 to 4.5 h of grazing a new allocation (Trevaskis et al., 2004; Gregorini et al., 2009a), grazing in the afternoon equated to a greater proportion of herbage DMI at greater OM and WSC concentrations in concordance with lesser CP and NDF concentrations.

CONCLUSIONS

Despite similar partitioning of N toward urine, given similar amounts of herbage allocation, a simple change in management practice such as allocating a strip later in the day resulted in moderate increases in N captured in milk and in milk solids yields in late-lactation dairy cows. Pasture management plays an important role in forage FA composition, and quantifying the composition of FA in herbage in response to grazing management strategies can aid in the design of grazing strategies to increase precursors of beneficial FA in dairy products. A greater concentration of precursor FA in a.m. herbage resulted in a greater concentration of RA and PUFA in milk, compared with cows on p.m. herbage. These benefits, plus a lower concentration of saturated FA, however, were realized in milk collected during the afternoon milking compared with the morning milking.

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