PERFORMANCE, NITROGEN UTILISATION AND GRAZING BEHAVIOUR FROM LATE-LACTATION DAIRY COWS OFFERED A FRESH ALLOCATION OF A RYEGRASS-BASED PASTURE EITHER IN THE MORNING OR IN THE AFTERNOON

Ronaldo Vibart, David Pacheco, Kate Lowe, Brent Barrett

AgResearch Grasslands Research Centre, Palmerston North

Abstract

The consumption of a more balanced fermentable carbon to nitrogen (C to N) ratio from fresh forages can potentially enhance dry matter (DM) intake, lactation performance, and N utilisation in dairy cows, particularly those in late lactation. Eighty lactating cows were used to examine the effects of allocating a morning (~0730 h, AM; two herds) vs. an afternoon (~1530 h, PM; two herds) fresh strip of a ryegrass (*Lolium perenne* L.)-based pasture on lactation performance, N utilisation, and grazing behaviour. The trial was conducted at the Massey University N^{o} 4 Dairy farm during April, 2010. Cows grazed on the same strip for a 24-hour period, and were offered the same daily DM allowance.

Pasture composition differed among treatments; herbage from the PM treatment had greater DM (22.7 vs. 19.9%; $P = 0.008$), organic matter (OM; 89.5 vs. 88.9%; $P < 0.001$) and water soluble carbohydrate (WSC; 10.9 vs. 7.6%; $P < 0.001$) concentrations, primarily at the expense of crude protein (CP; 20.5 vs. 22.2%; $P < 0.001$) and neutral detergent fibre (NDF; 48.8 vs. 50.4%; $P = 0.02$). Estimates of DM intake, from pre- and post-grazing herbage masses (HM), did not differ among treatments. Although daily milk yields were similar among treatments, trends towards greater milk fat, milk protein, and milksolids (MS) yields for cows on the PM treatment (1228 vs. 1132 g MS/cow; $P = 0.08$) were observed. Estimates of urinary N excretion (g/d) did not differ among treatments; urinary N concentration (g/L) was greater in samples collected during the morning (6.2 vs. 5.0 g/L, $P < 0.001$). Initial HM available (kg DM/ha) and instantaneous HM disappearance rates (kg DM/ha and per hour) did not differ among treatments, but fractional disappearance rates (0.56 vs. 0.74%/hour for AM vs. PM treatments, respectively) differed among treatments ($P = 0.04$). Despite similar partitioning of N towards urine, given similar amounts of herbage allocation, a simple change in management practice such as allocating a fresh strip later in the day resulted in moderate increases in N captured in milk and MS yields in late-lactation dairy cows.

Keywords: grazing, time of allocation, nitrogen utilisation, milk production, milksolids

Introduction

In New Zealand's pastoral dairying, nutritional needs of dairy cows are largely met by the supply of freshly grazed herbage from pasture, particularly from perennial ryegrass based pastures. Herbage from temperate pastures often increases its nutritive value during the day. Such increase in nutritive value has been largely attributed to dry matter (DM) and water soluble carbohydrates (WSC) accumulation (Gregorini *et al*., 2008), fibre and N dilution (Delagarde *et al*., 2000), enhanced digestibility (Abrahamse *et al*., 2009) and palatability (Fisher *et al*., 1999) along with enhanced biomechanical properties (i.e. reduced toughness)

(Gregorini *et al*., 2009b). Further, more than most other chemical components in the plant, WSC concentrations undergo diurnal fluctuation; as the day progresses, the rate of accumulation of WSC in the plant exceeds the rate of breakdown for growth and respiration (Fulkerson & Donaghy, 2001). These diurnal changes can potentially affect the temporal pattern of herbage intake and alter ingestive behaviour of grazing dairy cows (Gibb *et al*., 1998; Orr *et al*., 2001).

The consumption of a more balanced fermentable carbon to nitrogen (C to N) ratio from fresh forages can potentially enhance DM intake, lactation performance, and N utilisation in dairy cows, particularly those in late lactation (Cosgrove *et al*., 2007; Miller *et al*., 2001). Previous studies with lactating dairy cows have examined the impact of ryegrass-based diets with greater WSC concentrations on animal performance and N utilisation. It is important to note, however, that other chemical components are altered in association with increased WSC concentration, and these changes may partially contribute to animal responses. Dietary (*i.e.* plant) manipulation in these studies was largely via selective breeding (Cosgrove *et al*., 2007; Miller *et al*., 2001; Moorby *et al*., 2006; Tas *et al*., 2006; Taweel *et al*., 2006) and management-type approaches. In addition to dairy studies that were aimed at exploiting diurnal differences in fresh grass chemistry (Abrahamse *et al*., 2009; Trevaskis *et al*., 2004), most management-type means of dietary manipulation leading to improved nutritive value have included reduced N input from N fertilisation (Peyraud *et al*., 1997), prolonged regrowth periods (Delagarde *et al*., 2000) and a combination of the above (Rearte *et al*., 2005).

Among the grazing management strategies explored, diurnal accumulation of phosynthates accounted for consistent contrasts between WSC and CP concentrations in herbage (Cosgrove *et al*., 2009). These constrasts tend to match the daily pattern of grazing ruminants, which appear to maximize intake rate during the evening hours (Gibb *et al*., 1998). These findings provide for new opportunities to design grazing strategies that match greater intake rates with greater nutritive value. The objective of this study was to examine the effects of allocating a 24-hour strip of fresh herbage from a ryegrass-based pasture either in the morning or in the afternoon on lactation performance, N utilisation and grazing behaviour from late lactation dairy cows. It was hypothesised that cows with access to a new pasture allocation after the afternoon milking will produce more milk and milksolids.

Materials and Methods

Cows, Pastures and Treatments

Eighty lactating cows [mean ± SD 463 ± 46.8 kg initial liveweight (LW); 224 ± 21 initial days in milk, 12.2 ± 2.1 kg initial milk] were used in a short-term experiment to examine the effects of time of allocation of a fresh strip of a ryegrass-based pasture on lactation performance, N utilisation, and grazing behaviour. After blocking for milk, parity, and days in milk, cows were randomly assigned to either a morning allocation (AM; ~0730 h, following the morning milking) or an afternoon allocation (PM; ~1530 h, following the afternoon milking). Each allocation treatment included two herds of 20 cows each (40 cows per treatment). The experiment was conducted at the Massey University No 4 Dairy farm (40^o19’S, 157^o37E), located near Palmerston North, New Zealand, during April, 2010. The experiment included a period of adaptation (14 days) followed by a period of data collection (12 days). Liveweights were recorded once during the adaptation period, and twice during the data collection period. Cows were individually weighed (Tru-Test, Auckland, New Zealand) after morning milking. Seven paddocks, comprising a total area of 14.5 ha, were used throughout the experiment.

Data Collection and Calculations

Experimental paddocks were rotationally grazed with the aid of temporary electric fences. Cows grazed on the same strip for a 24-hour period, and were offered the same daily DM allowance, with a target daily DM intake (DMI) of ~16 kg per cow. The area offered daily per herd was calculated based on the daily DMI target (kg/cow), pre-grazing herbage mass (preHM) and post-grazing herbage mass (postHM) according to the following equation:

Area offered daily, ha = (number of cows per herd x target DMI)/(preHM – postHM)

Pre- and post-grazing HM were determined using an electronic rising plate metre (Farmworks Precision Farming Systems, Feilding, New Zealand), according to [HM, kg DM/ha = (158 x herbage height, cm) + 200]. The values recorded with the plate meter were the mean of approximately 50 readings taken randomly throughout the area to be allocated for grazing by each herd on a daily basis. Estimates of daily herbage intake were obtained by difference between pre- and post-grazing HM (Macoon *et al.*, 2003), according to the following equation:

DMI, kg DM/cow = ((preHM – postHM)/number of cows per herd) x area offered, ha

Milk yields were recorded automatically (Alpro, De-Laval) at every milking and milk samples were collected from each cow during the afternoon and following morning milking event three times (once during the adaptation period and twice during the data collection period) and analysed for fat, protein, lactose, and milk urea N (MUN) concentration, and somatic cell counts. Briefly, milk samples were collected from in-line samplers and immediately refrigerated at 4^{o}C until processing in the lab. Daily milksolids (MS) yield (fat + protein) was calculated using the concentration of fat and protein and the milk yields measured on the days of sample collection.

Estimates of N secreted in milk were obtained by [milk protein yield/6.38]. Milk fat, protein and lactose composition were obtained using a FT 6000 Fourier Transform infrared analyser (TestLink, Hamilton, New Zealand). Milk urea concentration was determined in milk supernatant by a urease-UV procedure, using a Flexor E clinical chemistry analyser (Vital Scientific, Dieren, The Netherlands) and a commercial diagnostic kit for urea (Roche Diagnostic NZ Ltd.) according to recommendations from the manufacturer.

In coincidence with the last two milk sampling periods, urine samples were collected immediately before, during, and after morning and afternoon milkings to estimate urinary N excretion. Spot urine samples were collected from all cows. If needed, a stimulatory massage was applied to those cows that did not urinate voluntarily. A representative sample of mid-stream urination was collected in a plastic container and immediately placed in ice until further processing and analyses. Urine was analysed for total N concentration using a combustion method (Carlo Erba Nitrogen Analyser; Carlo Erba, Italy) and creatinine concentration using a spectrophotometric assay, a compensated Jaffe reaction in a Hitachi 902 automatic analyzer (NZ Veterinary Pathology, Palmerston North, New Zealand).

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

a) From urinary creatinine in spot samples from pre-determined cows (n = 10 cows per herd), using a creatinine excretion factor of 21.9 mg/kg (Pacheco *et al.*, 2007), according to the following equation:

N in urine, g/d = ((21.9 x LW, kg)/urinary creatinine, mg/kg) x urine N, g/kg) (1)

b) From MUN concentration from pre-determined cows (n = 10 cows per herd) according to the equation of Jonker *et al*. (1998):

$$\text{N in urine, g/d} = 12.54 \times \text{MUN, mg/dL} \qquad (2)$$

Temporal grazing behaviour was recorded by trained observers during two 24-h periods, and grazing/non-grazing activities were recorded every 5 min from pre-assigned cows during daylight hours, and all cows during night time hours. For the purpose of this study, grazing was defined as cows in contact with the sward and engaged in acquiring herbage into the mouth, whereas non-grazing 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.

Corresponding herbage disappearance was assessed by using a rising plate metre at set time intervals (immediately before grazing, and every 30 minutes, up to 4 h after commencement of the main grazing events; ~0730 h for the AM herds and ~1530 h for the PM herds). Herbage mass disappearance rate was calculated from herbage mass measurements using a fractional disappearance model (Ørskov and McDonald, 1970) adapted to herbage disappearance (Gregorini *et al*., 2009a). Briefly, HM disappearance rate was obtained by fitting HM data (y) from each herd into the following equation:

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

where a = pre-grazing, available HM, kg DM/ha; b = potential HM disappearance, kg DM/ha; c = fractional disappearance rate of HM, %/h. Time was set at 0, 60, 120, 180, and 240 min after commencement of the main grazing events. Instantaneous HM disappearance rates (kg 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).

Concurrently with pasture disappearance assessment, herbage samples from the ryegrass-based pastures were collected immediately before grazing, and every 30 minutes, up to 4 h after commencement of the main grazing events. Samples were placed in ice, transported to the lab and stored frozen (-20^{o}C) and subsequently freeze-dried and ground to pass a 1 mm sieve. Chemical composition was obtained by near infrared reflectance spectroscopy (FeedTECH, Palmerston North, New Zealand). All pasture samples were analysed for ash, WSC, NDF, acid detergent fibre (ADF), CP, and lipid concentrations, along with organic matter digestibility (OMD) and metabolizable energy (ME) concentration.

Statistical Analysis

All data were analysed using the Statistical Analysis System package (SAS; version 9.1; SAS Institute Inc., Cary, North Carolina, USA). Pasture chemical composition was analysed using a general linear model (PROC GLM; SAS, 2002), with treatment (Table 1) and time of sample collection (Table 2) as fixed effects; mean comparisons were performed using the Tukey test. Lactation performance, milk composition and urinary N concentration data were analysed using a mixed model procedure (PROC MIXED; SAS, 2002) with repeated measurements over time. The models included fixed (treatment) and random (herd) effects. The models for milk and milk composition also included a covariate term, which comprised milk and milk composition data from two consecutive milkings during the adaptation period.

Data on time spent grazing was analysed using a mixed model procedure (PROC MIXED; SAS, 2002), whereas pasture disappearance data were analysed using a nonlinear procedure

(PROC NLIN; SAS, 2002). The model of Ørskov and McDonald (1970) was used to analyse the pasture disappearance data for each herd, using the Marquardt method to obtain the parameter estimates a, b, and c. Comparison of parameters was performed using a mixed model procedure (PROC MIXED; SAS, 2002) with herd as the experimental unit. Significance and trends were established at $P < 0.05$ and $P < 0.15$, respectively.

Results

Herbage Quantity and Nutritive Value

Herbage mass offered and refused during the entire experiment did not differ among treatments; mean (± SE) pre- and post-grazing HM were 2872 ± 147 kg DM/ha ($P = 0.39$) and 1831 ± 63 kg DM/ha ($P = 0.46$), respectively. Similarly, area offered daily to cows did not differ among treatments (0.393 ± 0.1 ha; $P = 0.26$). The chemical composition of the pasture varied by treatment (Table 1). Herbage offered to cows on the PM treatment 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, largely at the expense of CP (20.5 vs. 22.2%; $P < 0.001$) and NDF (48.8 vs. 50.4%; $P = 0.02$). To a lesser extent, lipid concentration was also reduced in PM herbage (2.8 vs. 3.1%; $P = 0.10$), whereas OMD and ME concentrations were similar among treatments (78.0 ± 0.57% and 11.4 ± 0.08 MJ ME/kg DM, respectively). Also, WSC concentrations increased throughout the day, attaining a maximum value at 1530 (10.3%; commencement of the main PM grazing event) (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 four 4 samples collected at each time point, whereas the treatment comparison (Table 1) was derived from 32 herbage samples analysed per treatment.

Table 1. Chemical composition of pasture allocated to cows either in the morning (AM) or in the afternoon (PM).

Treatment	Chemical composition[1], % DM							
	DM	OM	WSC	NDRF[2]	NDF	ADF	CP	Lipid
AM	19.9	88.9	7.6	5.7	50.4	25.7	22.2	3.1
PM	22.7	89.5	10.9	6.5	48.8	25.8	20.5	2.8
SE	0.70	0.09	0.45	0.54	0.45	0.26	0.25	0.10
$P \leq$	0.008	<0.001	<0.001	0.31	0.02	0.93	<0.001	0.10

[1]n = 32 herbage samples per treatment.
[2]Non-determined residual fraction = 100 – (ash + WSC + CP + NDF + lipid).

Table 2. Mean values of the diurnal pattern in chemical composition of a ryegrass (*Lolium perenne*)-based pasture during daylight hours.

Time of day, h	Chemical composition[1], % DM							
	OM	NFC	WSC	NDRF	NDF	ADF	CP	Lipid
0730	88.6	12.5	6.0[a]	6.5	51.5	26.5	21.5	3.0
1130	88.8	13.4	8.2[ab]	5.2	50.9	26.3	21.6	3.0
1530	88.9	14.8	10.3[b]	4.5	48.9	25.5	21.8	3.4
1930	89.3	15.2	10.1[b]	5.2	51.3	27.2	19.8	2.9
SE	0.19	1.22	0.95	1.63	1.50	0.79	0.69	0.12

[a,b]Mean values in the same column with different superscripts differ (p < 0.05)
[1]n = 4 herbage samples per time point.
[2]Non-fibre carbohydrates = 100 – (ash + CP + NDF + lipid).
[3]Non-determined residual fraction = 100 – (ash + WSC + CP + NDF + lipid).

Pasture Intake, Milk Production and Milk Composition

Estimates of DM intake, from pre- and post-grazing herbage masses (HM), did not differ among treatments (13.2 ± 0.76 kg DM/d) (Table 3). Daily milk yields were similar among treatments, but trends towards greater fat ($P = 0.05$) and protein ($P = 0.10$) yields resulted in a trend towards greater daily MS yields for cows on the PM treatment (1228 vs. 1132 g MS/cow; $P = 0.08$). Milk fat and milk protein concentrations did not differ among treatments. Similarly, MUN concentrations and somatic cell scores (SCS) did not differ among treatments.

Nitrogen Partition

Estimates of N intake (calculated from DMI and N concentration in herbage) tended to be greater for cows on AM ($P = 0.11$) whereas N output in milk tended to be greater for cows on PM ($P = 0.10$) (Table 4). 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 1 and 2, respectively). Urinary N concentrations (g/L) tended to be greater from cows on AM (5.87 vs. 5.35 g/L, SE = 0.26 g/L; $P = 0.11$); urinary N concentration was greater in samples collected during the morning (6.18 vs. 5.04 g/L, SE = 0.22 g/L; $P < 0.001$) (results not shown in Table).

Table 3. Effects of time of allocation of a fresh strip of pasture (AM, allotted at ~0730 h vs. PM, allotted at ~1530 h) on intake, milk production and milk composition. Cows grazed on the same strip for a 24-h period.

Treatment	Intake[1]	Milk	Milk fat		Milk protein		MS[2]	MUN[3]	SCS[4]
	kg/d	kg/d	%	g/d	%	g/d	g/d	mg/dL	
AM	13.4	12.2	5.15	627	4.15	505	1132	15.0	3.57
PM	13.0	13.1	5.27	684	4.19	545	1228	15.3	3.64
SE	0.76	0.27	0.061	5.9	0.022	9.2	17.3	0.27	0.08
$P \leq$	0.56	0.16	0.16	0.05	0.20	0.10	0.08	0.51	0.52

[1]Dry matter basis. [2]Milksolids (fat + protein yields). [3]Milk urea N, mg/dL.
[4]Somatic cell score = ((log10(SCC/1000) – 2)/log10(2)) + 3; SCC = Somatic cell count.

Table 4. Effects of time of allocation of a fresh strip of pasture (AM, allotted at ~0730 h vs. PM, allotted at ~1530 h) on estimated N intake, N in milk and N in urine. Cows grazed on the same strip for a 24-h period.

Treatment	N intake[1]	N in milk		N in urine[2]		N in urine[3]	
	g/d	g/d	Prop. Ni[4]	g/d	Prop. Ni[4]	g/d	Prop. Ni[4]
AM	475.0	79.2	0.17	220.0	0.46	192.1	0.40
PM	425.7	85.4	0.20	219.2	0.51	195.2	0.46
SE	26.8	1.4		6.9		6.2	
$P \leq$	0.11	0.10		0.93		0.60	

[1]N intake = (estimated DMI, kg/d x CP, %)/6.25.
[2]N in urine, calculated according to equation (1): N in urine, g/d = ((21.9 x LW, kg)/urinary creatinine, mg/kg) x urine N, g/kg).
[3]N in urine, calculated according to equation (2): N in urine, g/d = 12.54 x MUN, mg/dL.
[4]Expressed as a proportion of N intake.

Grazing Behaviour

Total time spent grazing was similar among treatments (510 ± 11.6 min; $P = 0.21$) (Table 5). Time spent grazing during the first 2 and 4 h after morning milking was greater for cows on AM ($P = 0.04$ and $P < 0.001$, respectively), whereas time spent grazing during the first 2 and 4 h after afternoon milking were similar among treatments. During the grazing behaviour observation periods, initial HM available (kg DM/ha), potential HM disappearance (kg DM/ha) and instantaneous HM disappearance rates (kg DM/ha per hour) did not differ among treatments (Table 6). Fractional disappearance rates, however, were greater for cows on PM (0.74 vs. 0.56%/hour; $P = 0.04$).

Table 5. Effects of time of allocation of a fresh strip of pasture (AM, allotted at ~0730 h vs. PM, allotted at ~1530 h) on time spent grazing (min). Cows grazed on the same strip for a 24-h period.

Treatment	Grazing time				
	Total, min/d	First 2 hours, min		First 4 hours, min	
		Post-am[1]	Post-pm[2]	Post-am[1]	Post-pm[2]
AM	525	117	120	196	189
PM	495	86	120	110	194
SE	11.6	8.1	1.8	9.9	8.0
$P \leq$	0.21	0.036	0.95	<0.001	0.67

[1]After morning milking.
[2]After afternoon milking.

Table 6. Effects of time of allocation of a fresh strip of pasture (AM, allotted at ~0730 h vs. PM, allotted at ~1530 h) on the dynamics of herbage mass (HM) disappearance during the first 4 h of the main grazing event. Cows grazed on the same strip for a 24-h period.

Treatment	Parameters[1]			Herbage mass disappearance rate[2] at				
	a	b	c	0	60	120	180	240
AM	3352	1069	0.56	589	324	184	108	65
PM	3256	970	0.74	694	333	163	82	42
SE	84.6	59.2	0.08	69.1	26.6	20.5	17.7	14.3
$P \leq$	0.43	0.24	0.04	0.33	0.83	0.50	0.33	0.29

[1]HM disappearance rates obtained by fitting HM data (y) from each herd into the equation [$y = a - b(1 - \exp(-c \times time))$] (Ørskov and McDonald, 1970) where a = pre-grazing, available HM, kg DM/ha; b = potential HM disappearance, kg DM/ha; c = fractional disappearance rate of HM, %/h.
[2]HM disappearance rates, kg DM/ha per h, estimated at 0, 60, 120, 180, and 240 min after commencement of the main grazing events.

Discussion

The aim of this short-term study was to examine the effects of time of allocation of a daily strip of ryegrass-based pasture on aspects of lactation performance, N utilisation and grazing behaviour from late lactation dairy cows. Although daily strip grazing is a commonly adopted grazing practice in modern pastoral dairying, the role of timing of herbage allocation has only received limited attention; information on the combined effects of lactation performance, N utilisation and grazing behaviour is relatively scarce, as reported by Abrahamse *et al*. (2009). In the present experiment, increased nutritive value from allocating a fresh strip of forage after the afternoon milking, rather than after the morning milking, was associated with a moderate improvement in MS yields.

Herbage Quality

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* L.; Trevaskis *et al*., 2004), phalaris (*Phalaris aquatica* L.; Ciavarella *et al*., 2000), tall fescue (*Lolium arundinaceum* Schreb; Fisher *et al*., 1999), coksfoot (*Dactylis glomerata* L.; Griggs *et al*., 2005), wheat (*Triticum aestibum* L.; Gregorini *et al*., 2008) and lucerne (*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 occur particularly in the upper layers of the sward, associated with processes of intense photosynthesis and gas exchange (Delagarde *et al*., 2000). In the present experiment, DM concentration increased by 14% by offering PM vs. AM forage. A similar difference (19.6%) was reported by Gregorini *et al*. (2008) for wheat sampled at either 0800 or 1900 h. 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 by Gregorini *et al*. (2008) (88.6 vs. 83.0%).

Although not statistically significant, NDF concentration followed a similar diurnal pattern to that of Gregorini *et al*. (2008) for wheat and Burns *et al*. (2005) for lucerne, starting with the greatest value at 0830, reaching the lowest value at 1530, and increasing again at 1930 h, similar to that of the initial NDF concentration. An inverse relationship was followed by WSC and neutral detergent solubles (100 – NDF; results not shown), and this pattern was presumably attributed with 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.

Changes in nutritive value of temperate grasses as the day progresses are largely attributable to an increase in the accumulation of photosynthates, largely WSC, as reported in this study. An associated passive dilution of NDF and CP concentrations occurs concurrently, a common characteristic reported in most of the above mentioned studies. Results reported herein sustain this premise; the herbage allocated to cows after the afternoon milking was of greater nutritive value than that allocated after the morning milking.

Intake, Lactation Performance and N Partition

Daily herbage intake was similar among treatments; these findings are in agreement with those reported by Orr *et al*. (2001) and Abrahamse *et al*. (2009) for dairy cows in mid-lactation grazing a perennial ryegrass-based pasture, and by Gregorini *et al*. (2008) for Angus heifers grazing a monoculture of wheat. These authors found no difference in daily herbage DMI when morning vs. afternoon allocation of a fresh strip of pasture was compared.

In the present study, although estimates of 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 PM vs. AM forage. Consistent with our findings, Orr *et al*. (2001) reported a 5% increase in milk yields for cows moved to a fresh 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 PM, 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 PM, largely attributable to greater milk fat concentrations.

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 observed in MUN (Gustafsson and Palmquist, 1993), suggesting that an improved balance in the supply of energy from non-structural carbohydrates and N leading to reduced ruminal ammonia N concentrations will influence excretion of urea N in milk. However, the improved WSC to CP ratio provided by the PM herbage may not have been sufficient to elicit a significant response in N excretion, as was evidenced by similar MUN excretion and estimates of urinary N losses (g/d). Nonetheless, the amount of N secreted in milk tended to be greater for cows on PM, and N utilisation efficiency (N in milk expressed as a proportion of N intake) increased by 18% by offering PM vs. AM forage. These findings, along with trends towards reduced N intake and reduced urinary N concentrations (g/L), suggest an overall improvement in N utilisation from cows on PM. These findings are in agreement with those reported by Brito *et al*. (2008) for dairy cows in late lactation fed lucerne baleage that was harvested either in the morning or in the afternoon.

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 GHG 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.

Grazing Behaviour

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 unsupplemented 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. In the present study, total grazing time by cows on the AM 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 AM spent more time grazing during the first four hours after morning milking, but the time spent grazing after the first four hours after afternoon milking remained similar among treatments. Although estimates of daily DMI were similar among AM and PM cows, the above findings, along with a different herbage disappearance rate (%/h), suggest that the temporal pattern of intake differed considerably. Cows given a new allocation in the afternoon obtained more of their DMI in the evening, when the DM and WSC concentrations were greater.

In similar experimental setups, Orr *et al*. (2001) and Abrahamse *et al*. (2009) reported a similar intake pattern for dairy cows allocated to either AM or PM herbage. These authors reported that cows allocated to AM herbage tended to have lower bite masses in the afternoon, but greater effects (even lower bite masses) were observed for cows on PM grazing in the morning. These findings are consistent with both 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 PM 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 AM) or increased biting rate (i.e. cows on PM).

Assuming that more than 70% of the daily intake of herbage from pasture occurs within the first 3 to 4.5 hours of grazing a new allocation (Trevaskis et al., 2004), grazing in the afternoon equated to a greater proportion of herbage DM intake at greater OM and WSC concentrations in concordance with lesser CP and NDF concentrations. Despite similar partitioning of N towards urine, given similar amounts of herbage allocation, a simple change in management practice such as allocating a fresh strip later in the day resulted in moderate increases in N captured in milk and in milksolids yields in late-lactation dairy cows.

Acknowledgements
Project funded by Pastoral 21 (Feeds platform) and the Foundation for Research, Science and Technology (Contract 10X0604). The authors wish to thank the staff at No. 4 Dairy Farm at Massey University for assistance during the trial, and Gerald Cosgrove and Brian Devantier for their valuable input.

References

Abrahamse PA, Tamminga S, Dijkstra J, 2009. Effect of daily movement of dairy cattle to fresh grass in morning or afternoon on intake, grazing behaviour, rumen fermentation and milk production. Journal of Agricultural Science 147:721-730.

Brito AF, Tremblay GF, Bertrand A, Castonguay Y, Belanger G, Michaud R, Lapierre H, Benchaar C, Petit HV, Ouellet DR, Berthiaume R, 2008. Alfalfa cut at sundown and harvested as baleage improves milk yield of late-lactation dairy cows. Journal of Dairy Science 91:3968-3982.

Burns JC, Mayland HF, Fisher DS, 2005. Dry matter intake and digestion of alfalfa harvested at sunset and sunrise. Journal of Animal Science 83:262-270.

Ciavarella TA, Simpson RJ, Dove H, Leyry BJ, Sims IM, 2000. Diurnal differences in the concentration of water-soluble carbohydrates in *Phalaris aquatica* L. pasture in spring, and the effect of short-term shading. Australian Journal of Agricultural Research 51:749-756.

Cosgrove GP, Burke JL, Death AF, Hickey MJ, Pacheco D, Lane GA, 2007. Ryegrasses with increased water soluble carbohydrate: evaluating the potential for grazing dairy cows in New Zealand. Proceedings of the New Zealand Grassland Association 69:179-185.

Cosgrove GP, Koolaard J, Luo D, Burke JL, Pacheco D, 2009. The composition of high sugar ryegrasses. Proceedings of the New Zealand Grassland Association 71:187-193.

Delagarde R, Peyraud JL, Delaby L, Faverdin P, 2000. Vertical distribution of biomass, chemical composition and pepsin-cellulase digestibility in a perennial ryegrass sward: Interaction with month of year, regrowth age and time of day. Animal Feed Science and Technology 84:49-68.

Fisher DS, Mayland HF, Burns JC, 1999. Variation in ruminant preference for tall fescue hays cut at sundown or sunup. Journal of Animal Science 77:762-768.

Fulkerson WJ, Donaghy DJ, 2001. Plant-soluble carbohydrate reserves and senescence - key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. Australian Journal of Experimental Agriculture 41:261-275.

Gibb MJ, Huckle CA, Nuthall R, 1998. Effect of time of day on grazing behaviour by lactating dairy cows. Grass and Forage Science 53:41-46.

Gibb MJ, 2006. Grassland management with emphasis on grazing behaviour. *In*: Fresh Herbage for Dairy Cattle (Ed. Dijkstra J, Tamminga S), pp. 141-157. Dordrecht, The Netherlands.

Griggs TC, MacAdam JW, Mayland HF, Burns JC, 2005. Nonstructural carbohydrate and digestibility patterns in orchardgrass swards during daily defoliation sequences initiated in evening and morning. Crop Science 45:1295-1304.

Gregorini P, Gunter SA, Beck PA, 2008. Matching plant and animal processes to alter nutrient supply in strip-grazed cattle: Timing of herbage and fasting allocation. Journal of Animal Science 86:1006-1020.

Gregorini P, Clark CEF, Jago JG, Glassey CB, McLeod KLM, Romera AJ. 2009a. Restricting time at pasture: Effects on dairy cow herbage intake, foraging behavior, hunger-related hormones, and metabolite concentration during the first grazing session. Journal of Dairy Science 92:4572-4580.

Gregorini P, Soder KJ, Sanderson MA, Ziegler GR, 2009b. Toughness, particle size and chemical composition of meadow fescue (*Festuca pratensis* Hud.) herbage as affected by time of day. Animal Feed Science and Technology 151:330-336.

Gustafsson AH, Palmquist DL, 1993. Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. Journal of Dairy Science 76:475-484.

Jonker JS, Kohn RA, Erdman RA. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. Journal of Dairy Science 81:2681-2692.

Ledgard SF, Welten BG, Menneer JC, Betteridge K, Crush JR, Barton MD, 2007. New nitrogen mitigation technologies for evaluation in the lake Taupo catchment. Proceedings of the New Zealand Grassland Association 69:117-121.

Macoon B, Sollenberger LE, Moore JE, Staples CR, Fike JH, Portier KM, 2003. Comparison of three techniques for estimating the forage intake of lactating dairy cows on pasture. Journal of Animal Science 81:2357-2366.

Miller LA, Moorby JM, Davies DR, Humphreys MO, Scollan ND, MacRae JC, Theodorou MK, 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. Grass and Forage Science 56:383-394.

Moorby JM, Evans RT, Scollan ND, MacRae JC, Theodorou MK, 2006. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in dairy cows in early lactation. Grass and Forage Science 61:52-59.

Orr RJ, Rutter SM, Penning PD, Rook AJ, 2001. Matching grass supply to grazing patterns for dairy cows. Grass and Forage Science 56:352-361.

Ørskov ER, McDonald I, 1970. The estimation of protein degradability in the rumen from incubation measurement weighed according to the rate of passage. Journal of Agricultural Science 92:499-508.

Pacheco D, Burke JL, Death AF, Cosgrove GP, 2007. Comparison of models for estimation of urinary nitrogen excretion from dairy cows fed fresh forages. *In*: Meeting the Challenges for Pasture-Based Dairying. Proceedings of the 3rd Australasian Dairy Science Symposium, pp. 417-422. Melbourne, Australia.

Pacheco D, Lowe K, Hickey MJ, Burke JL, Cosgrove GP, 2010. Seasonal and dietary effects on the concentration of urinary N from grazing dairy cows. *In*: Meeting the Challenges for Pasture-Based Dairying. Proceedings of the 4th Australasian Dairy Science Symposium, pp. 68-73. Lincoln University, Christchurch, New Zealand.

Peyraud JL, Astigarraga L, Faverdin P, 1997. Digestion of fresh perennial ryegrass fertilized at two levels of nitrogen by lactating dairy cows. Animal Feed Science and Technology 64:155-171.

Rearte DH, 2005. New insights in the nutritive value of grass. *In*: Utilisation of Grazed Grass in Temperate Animal Systems. Proceedings of a Satellite Workshop of the 20th International Grassland Congress (Ed. Murphy JJ), pp. 49-59. Cork, Ireland.

Tas BM, Taweel HZ, Smit HJ, Elgersma A, Dijkstra J, Tamminga S, 2006. Effects of perennial ryegrass cultivars on milk yield and nitrogen utilization in grazing dairy cows. Journal of Dairy Science 89:3494-3500.

Taweel HZ, Tas BM, Smit HJ, Elgersma A, Dijkstra J, Tamminga S, 2006. Grazing behaviour, intake, rumen function and milk production of dairy cows offered *Lolium perenne* containing different levels of water-soluble carbohydrates. Livestock Science 102:33-41.

Trevaskis LM, Fulkerson WJ, Nandra KS. 2004. Effect of time of feeding carbohydrate supplements and pasture on production of dairy cows. Livestock Production Science 85:275-285.