

## Milk production and urination behaviour of dairy cows grazing diverse and simple pastures

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### Abstract

Milk production and urination patterns were measured for mid-lactation dairy cows grazing a simple perennial ryegrass-white clover pasture (n = 15) and a more diverse pasture (n = 15) containing herb (chicory and plantain) and legume (lucerne) species in addition to the perennial ryegrass and white clover. Milk solids production was similar for simple (2.09 kg MS/cow/d) and diverse (1.94 kg MS/cow/d) pasture. Urination volume and frequency were similar for simple (2.0 L/urination, 11.6 urinations/cow/d) and diverse (2.2 L/urination, 15.0 urinations/cow/d) pasture. Urine-nitrogen (N) concentration was 20% lower for diverse (4.9 g N/L) than simple (6.1 g N/L) pasture. Diverse pastures may offer benefits for reducing the environmental impact of dairy farming by reducing the N loading of urine patches.

**Keywords:** diverse; milk production; pasture; simple; urination

### Introduction

There is increased pressure to reduce the environmental impact of dairy farming (Ministry for the Environment 2011). For New Zealand, nitrogen (N) management is an important issue, due to its potential impact through nitrate leaching on water quality in aquifers, lakes and rivers (Ledgard et al. 1999). Nitrogen from urine patches is a major contributor to N leaching, (Di & Cameron 2007; Selbie et al. 2015). Therefore, strategies that manipulate urine volume, frequency and N concentration are important for mitigating the environmental impacts. One option to achieve this is to use variation within and between forages in nutritive characteristics (water-soluble carbohydrate, crude protein), mineral profiles and secondary plant components, to reduce urinary-N excretion, or divert dietary N away from urine (Pembleton et al. 2015; Totty et al. 2013). Recent analyses have shown an important role for more diverse pastures containing additional herbs (chicory, *Cichorium intybus*) and plantain, *Plantago lanceolata*) to the standard perennial ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) mixture used on dairy farms. Woodward et al. (2012) reported lower urine-N concentration and N excretion in diverse than simple perennial ryegrass-white clover pastures, a result that reflected large differences in N intake. In a study where N intake was matched, Totty et al. (2013) reported lower measured urine-N concentration and estimated urinary-N excretion in diverse than simple perennial ryegrass-white clover pastures. However, before farmers have confidence in the use of diverse pastures to reduce N loading in urine patches, more studies are needed to confirm the consistency of the response and the effect on milk production. Further, it is important to ascertain effects on urination patterns, as in conjunction with urine-N concentration, these determine urine-patch N loading (Selbie et al. 2015).

The objective of this experiment was to compare milk production and urination behaviour of dairy cows offered simple perennial ryegrass-white clover pasture and diverse pasture containing additional herbs (chicory and plantain) and legumes (lucerne, *Medicago sativa*).

### Materials and methods

#### Experimental site and treatments

The experiment was conducted at the Lincoln University Research Dairy Farm with the approval of the Lincoln University Animal Ethics Committee (AEC 523). Thirty Friesian x Jersey cows were blocked according to pre-experimental live weight ( $475 \pm 11.1$  kg), milk solids production ( $1.88 \pm 0.6$  kg MS/cow/d), days in milk ( $112 \pm 2.9$  days), and age ( $4.2 \pm 0.3$  years) (all mean  $\pm$  sem) and assigned from within blocks to graze one of two pasture treatments: simple (perennial ryegrass and white clover) (n=15 cows) and diverse (perennial ryegrass, white clover, plantain, chicory, and lucerne) (n=15 cows). Cows grazed pastures for a period of nine days from 10 to 19 December. Before the start of the trial, all cows rotationally grazed together on a perennial ryegrass-white clover pasture.

The pastures were established in October 2013, and rotationally grazed with dairy cows prior to the experiment starting. Pasture was irrigated with a centre-pivot irrigator. Pastures were grazed to a constant height (c. 4.5 cm), and fertilized with 50 kg N/ha as urea 28 days before the trial commenced. Cows were milked twice daily (approximately 0700 and 1530 h) and offered a target daily allowance after each morning milking of 18 kg DM/cow/day above 1500 kg DM post-grazing herbage mass. Daily herbage allocation during the experiment were based on previously derived calibration equations between herbage mass and pasture height for diverse and simple pastures (Engelbrecht et al. 2014), with the area grazed controlled by temporary electric fencing. Each daily allocation was back-fenced to prevent grazing of residual regrowth. Cows had *ad lib* access to water through a portable trough connected to a flow meter.

#### Herbage measurements

At least 50 compressed pasture height measurements were taken daily pre- and post-grazing using a calibrated rising plate meter (RPM, Jenquip, Filip's EC 09, Electronic Folder plate meter). The pre-grazing measurements were taken in the area estimated to be allocated in the next forage

allocation. Calibration measurements were collected from simple ( $n = 27$ ) and diverse ( $n = 45$ ) pastures by cutting  $0.2 \text{ m}^2$  quadrats to ground level before and after grazing during the experiment. Two RPM measurements were recorded in each quadrat before all herbage was harvested to ground level, oven-dried at  $65^\circ\text{C}$  for 48 h and dry weight determined. Linear and curvilinear relationships between herbage mass and pasture height were compared and best-fit equations (lowest  $r^2$ ) were fitted to the data. The calibration equations for each pasture were: simple ( $\text{kg DM/ha} = 401 \times \text{cm} - 215$ ,  $r^2 = 0.80$ ), and diverse ( $\text{kg DM/ha} = 2760 \times \ln(\text{cm}) - 2816$ ,  $r^2 = 0.70$ ). Using equations derived from the experimental data set and grazing areas, the actual herbage allocations were 18.5 and 20.1  $\text{kg DM/cow/day}$  above 1500  $\text{kg DM/ha}$  for simple and diverse pastures, respectively. Apparent group DM intake of cows was calculated from herbage disappearance between pre- and post-grazing herbage and area allocated.

Herbage samples (around 200 g) were collected daily pre-grazing to ground level and split into two subsamples. One subsample was sorted into sown grass, herbs, legumes, weeds and dead material before drying at  $65^\circ\text{C}$  for 48 hours and weighing. The proportion of each species on a DM basis was then determined. The second sample of mixed herbage was oven-dried at  $65^\circ\text{C}$  for determination of DM%, before being ground through a 1-mm sieve, and scanned by near infra-red spectrophotometer (NIRS, NIRSystems 5000, Foss, Maryland, USA) to determine crude protein (CP), digestible organic matter (DOMD), water-soluble carbohydrate (WSC), acid-detergent fibre and neutral-detergent fibre (NDF) (Lincoln University Analytical Laboratory). Metabolisable energy (ME) was calculated as  $\text{MJ ME/kg DM} = 0.16 \times \text{DOMD}$  (CSIRO, 2007). The ME for both pastures was calculated using this equation. A herbage sample collected to ground level after grazing daily each day and was analysed for CP content to determine the CP content of the herbage consumed.

#### *Animal measurements*

On two occasions (day 6 and 10), urination events for all cows in each treatment were recorded by visual assessment between 0830 and 1330 h by noting the cow which urinated and the time of urination. Spot samples of urine and faeces were collected on day 5 and 9 from all cows after the morning and afternoon milking. Urine and faecal samples were analysed for N% and DM% as described by Miller et al. (2012). Diurnal variation in urine-N concentration has previously been reported (Bryant et al. 2013). Thus, it is recognized that spot sampling of urine will provide a conservative estimate of urine-N concentration. Individual milk yield was measured daily with an automated system (DeLaval Alpro Herd management System, DeLaval, Tumba Sweden). Milk samples were collected for every cow at the morning and afternoon milking on day 5 and 9 to determine milk composition. Milk composition was analysed by the laboratory of Livestock Improvement Corporation Ltd (Christchurch, New Zealand) to determine milk fat, protein and lactose by MilkoScan (Foss Electric, Hillerod, Denmark).

A urine meter harness (see Ravera et al. (2015) for description) was worn by twelve randomly selected cows in each treatment over six runs of up to 24 hours from days 4 to day 9 of trial. Briefly the harness works by attaching a flow meter to the vulva of the cow which transmits pulse signals to a data logger whenever the cow urinates. Non-urination movement of the flow meter resulted in a number of readings of small volumes which Ravera et al. (2015) was able to filter by omitting data from event durations of less than 4 seconds. However in the current study using lactating cows, extended bouts of walking resulted in long non-urination events with minimal volume recorded (e.g. a reading of 60 seconds recording 0.02 L). Consequently, analysis of data excluded events of  $< 0.08 \text{ L/s}$  which was approximately equivalent to filtering out events that resulted in a volume record of  $< 0.1 \text{ L}$ . On occasions, the meters failed, either through wires breaking or the harness becoming unstuck from the cow. This left eight cows from which data could be used between evening and morning milking and only six cows providing data over a 24 hour period. Each water trough was fitted with a flow meter and water intake of each treatment group measured on daily basis.

#### *Statistical analysis*

The effects of pasture type on milksolids production, milk composition, and urination behaviour was analysed by ANOVA (GenStat 15.1 VSN International LTD. 2012), with cow as random effect and pasture type as a fixed effect. In each case, the experimental unit was data averaged for individual cows over sampling days. No statistical analysis was carried out on herbage samples or apparent DM intake as they were based on samples collected from each daily allocation within the same paddock.

## **Results**

### *Herbage*

Herbage characteristics and composition were very similar for both pastures (Table 1). The key difference was the herb content in the diverse pasture which accounted for 15% of the DM to ground level. The lack of herb in the simple pasture was offset by a small increase in the clover, grass and weed content in the simple pasture. In the diverse pasture, lucerne represented a third of the total legume. Due to a high legume content in both pastures ( $>24\%$ ), there was no difference in CP content of pre-grazing herbage. However, the concentration of CP post-grazing was lower in the simple (13.2%) than diverse (17.2%) pasture resulting in a herbage CP content in the selected diet of 228 and 192  $\text{g CP/kg DM}$  in the simple and diverse pasture, respectively.

### *Animal*

Apparent DM intake per daily grazing area was similar between simple ( $15.3 \pm 2.1 \text{ kg DM/cow/d}$ ) and diverse ( $16.2 \pm 1.4 \text{ kg DM/cow/d}$ ) pastures. Pasture treatment also had no significant effect on milk yield, milk composition or milksolids production, although milk urea N was higher for simple pasture (Table 2). Urination frequency and volumes

**Table 1** Mean herbage characteristics ( $\pm$  sem, n = 9) and chemical composition of simple and diverse pastures sampled to ground level.

	Simple	Diverse
Pre-grazing		
Compressed sward height (cm)	9.6 $\pm$ 1.4	10.5 $\pm$ 0.6
Herbage mass (kg DM/ha)	3625 $\pm$ 555	3634 $\pm$ 152
Legume (%)	28.6 $\pm$ 5.2	24.9 $\pm$ 5.1
Grass (%)	58.4 $\pm$ 7.2	54.9 $\pm$ 7.2
Herb (%)	0 $\pm$ 0	15.1 $\pm$ 3.9
DM %	12.8 $\pm$ 2.3	14.4 $\pm$ 1.6
CP (g/kg DM)	186 $\pm$ 9.3	182 $\pm$ 9.7
WSC (g/kg DM)	202 $\pm$ 19.5	217 $\pm$ 15.5
NDF (g/kg DM)	381 $\pm$ 18.2	394 $\pm$ 13.2
ADF (g/kg DM)	245 $\pm$ 7.2	245 $\pm$ 6.2
ME (MJ ME/kg DM)	11.8 $\pm$ 0.1	12.0 $\pm$ 0.1
Post-grazing		
Compressed sward height (cm)	4.5 $\pm$ 0.3	5.6 $\pm$ 0.2
Herbage mass (kg DM/ha)	1605 $\pm$ 251	1903 $\pm$ 119

CP, crude protein; WSC, water-soluble carbohydrates; NDF, neutral-detergent fibre; ADF, acid-detergent fibre; ME, metabolisable energy

**Table 2** Mean milk yield and milk composition (n = 15) of dairy cows grazing simple and diverse pastures. LSD = least significant difference ( $\alpha$  = 0.05).

	Simple	Diverse	LSD	P value
Milk yield (kg/cow/d)	22.4	21.1	1.65	0.17
Milk fat (%)	5.37	5.23	0.52	0.34
Milk protein (%)	4.03	4.08	0.18	0.54
Milk Lactose (%)	5.08	5.04	0.07	0.31
Fat (kg/cow/d)	1.19	1.08	0.13	0.10
Protein (kg/cow/d)	0.89	0.85	0.07	0.28
Milksolids (kg/cow/d)	2.09	1.94	0.18	0.13
Milk urea N (mmol/L)	12.68	8.64	0.83	<0.01

**Table 3** Mean urination behaviour, and urine and faecal characteristics of dairy cows grazing simple and diverse pastures. LSD = least significant difference ( $\alpha$  = 0.05). n = 15 for urine-N concentration, faecal-N concentration and faeces DM%.

	Simple	Diverse	LSD	P value
Urine				
Volume (L/urination) <sup>1</sup>				
0700 – 1400 h	1.8 (47)	2.3 (90)	1.3	0.44
1530 – 0630 h	2.4 (43)	2.2 (61)	0.9	0.70
Frequency (urinations/cow) <sup>2</sup>				
0830 – 1330 h	1.3 (15)	1.6 (15)	0.6	0.43
1530 – 0630 h	10.3 (4)	13.4 (4)	4.3	0.13
N concentration (g N/L)	6.1	4.9	0.7	<0.01
Faeces				
DM %	12.5	11.4	3.7	0.54
N concentration (g N/kg DM)	3.6	3.3	0.2	<0.01

<sup>1</sup>Means from urine meter, values in parenthesis are total number measurements. <sup>2</sup> Values in parenthesis represent the number of cows on which data was recorded. Morning values are from visual assessment and do not include events which occur in races or yards.

are shown in Table 3. There was large variation among animals in the volume and frequency of each urination. For the six cows which had at least five readings, the minimum volume per urination ranged from 0.5 to 1.6 L, while the maximum volume per urination ranged from 2.6 to 11.2 L. The mean urine volume, adjusted for number of urinations in morning and afternoon was 2.0 and 2.2 L for simple and diverse, respectively. The frequency of urination from the same six cows ranged from 11 to 31 urinations/cow/day. The mean daily frequency of urination from combined visual assessment and meter records was 12.0 and 14.9 urinations/cow/d for simple and diverse pasture, respectively. Of the small number of cows which wore the harness for a 24 hr period (n = 2 and 4 cows, for simple and diverse respectively), the total mean daily urine volume was 40.5  $\pm$  17.7 L and 37.3  $\pm$  1.4 L /cow/d for diverse and simple pasture, respectively. The N concentration of urine was 1.2 g N/L lower for cows grazing diverse than simple pasture (Table 3). Faecal-N concentration was higher for cows grazing simple than diverse pasture. Group-water intake of dairy cows was similar for simple (26 L/cow/d) and diverse (28 L/cow/d) pasture.

## Discussion

### *Apparent DM intake, milk production and milk composition*

Milk yield, milk composition and milksolids production were similar for cows offered a simple binary mixture of perennial ryegrass and white clover and a more diverse pasture also containing chicory, plantain and lucerne. These results are consistent with previous studies which found similar milksolids production for dairy cows grazing simple and diverse pastures (Engelbrecht et al. 2014; Soder et al. 2006; Totty et al. 2013). The lack of response in the current study is most likely because negligible differences were detected in apparent DM intake or the ME content of the pasture on offer. However, caution is needed to interpret the apparent DM intake and pasture nutritive value data as samples were collected from each daily allocation within the same paddock and no statistical analysis was possible.

### *Urine volume and frequency*

The mean volume of each urination (1.8 to 2.4 L/urination) and mean frequency of urination (11.6 to 15.0 urinations/cow/d) observed in this study are within the ranges described for dairy cattle by Selbie et al. (2015) in a review of urination behaviour. However, a feature of the results from this study was that both urine volume and frequency were highly variable. Urine volume ranged from 0.5 to 11.2 L/urination, while urine frequency ranged from 11 to 31 urinations per day. High variability in urine volume has also been noted previously (Selbie et al. 2015). These results have implications for prediction of nitrate leaching. In particular, they indicate that N leaching estimated for the whole paddock, but based on lysimeter experiments and scaling using mean urine characteristics may be incorrect (see also Betteridge et al. (2013)).

There were negligible differences in the volume of each urination, the frequency of urination, or total urine volume between simple and diverse pasture (Table 3). Differences in urine volume and frequency between diets have been related to water intake, forage composition, mineral composition and the presence of secondary plant compounds (Selbie et al. 2015; Stewart 1986). In this study, drinking water intake was similar (mean = 27 L/cow/d) between pastures, and consistent with previous reports of drinking water intake (26.3 L/cow/d) of lactating dairy cows (McLeod et al. 2009). Based on the DM% values of forages (Table 1) and the apparent DM intake values (Table 2), estimated water intake from forage was approximately 100 L/cow/d for both simple and diverse pasture. It was hypothesised that there may be a diuretic effect of herbs which would increase urine volume of animals on these diets. While this cannot be discounted from the small data set in this study, which showed numerically higher urination frequency and volume under diverse pastures, the lack of apparent effect on urination may reflect small differences in water intake. Also, the percentage of herb in the DM of diverse pastures was low (<15%), and probably insufficient to lead to differences in urination behaviour on the diverse pasture.

Although no effect of pasture type on urine volume and frequency was observed, urine N concentration was affected, being 1.2 g N/L lower for diverse than simple pasture (Table 3). Further MUN and faecal-N concentration were lower in diverse than simple pastures. The lower urine N concentration, MUN and faecal-N concentration for diverse than simple pasture has been observed previously (Totty et al. 2013) and most probably reflects the lower apparent N intake for diverse (497 g N/cow/d) than simple (551 g N/cow/d) pasture. The implication of the lower urine N concentration is that, given similar volume per urination between pastures, N loading in the urine patch will be lower in the diverse pasture. As N loading in the urine patch is a key determinant of nitrate leaching (Di & Cameron 2007), this result suggests that diverse pastures have potential to reduce nitrate leaching.

## Conclusion

Our results demonstrate a role for use of diverse pastures containing herbs as a mitigation strategy to reduce the environmental impact of dairy farming. By providing a diverse compared to simple pasture in mid-lactation, there was no decline in milk production, but urine-N concentration and apparent N loading in the urine patch were reduced.

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