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## Milk production and urinary nitrogen excretion of dairy cows grazing perennial ryegrass-white clover and pure plantain pastures

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### Abstract

Milk production and urinary nitrogen (N) concentration were measured in late lactation dairy cows grazing a perennial ryegrass-white clover pasture, pure plantain and an area that is comprised of 50% perennial ryegrass-white clover and 50% pure plantain by ground area (50-50 pasture-plantain), (n=12). Milksolids production was greater (P=0.01) for cows grazing plantain (1.67 kg MS/cow/d) than those grazing pasture (1.50 kg MS/cow/d), with cows grazing 50-50 pasture-plantain intermediate (1.60 kg MS/cow/d). Urine-N concentration was 56% lower (P<0.001) for plantain (2.4 g N/L) and 33% lower for 50-50 pasture-plantain (3.6 g N/L) than pasture (5.4 g N/L). Plantain may offer environmental benefits to dairy systems by reducing the urinary N concentration deposited on the soil from grazing cows in late lactation.

**Keywords:** herb; *Lolium perenne*; nitrate; *Plantago lanceolata*; *Trifolium repens*

### Introduction

Reducing the environmental impacts of the dairy industry in New Zealand has become a key focus, with increasing interest surrounding nitrogen leaching issues (Bryant et al. 2007; Woodward et al. 2012). Regional Councils throughout New Zealand have been developing regulations that place a limit on the amount of nitrate-N leaching from agricultural land. Nitrogen from urine patches is a major contributor to N leaching (Di & Cameron 2007). This is due to a large discrepancy between the N content of grazed forages, and the N requirement of the animals; N in excess of an animal's requirement is excreted, primarily in the urine (Tammenga 1992). Mitigation strategies have utilised variation in the chemical composition (water-soluble carbohydrate, crude protein), mineral profile and secondary plant compounds in forages, to reduce urinary-N excretion, or divert dietary N away from urine. Previous experiments (Woodward et al. 2012; Totty et al. 2013; Edwards et al. 2015) achieved this by incorporating alternative pasture species including plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*) and additional legumes such as red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*) into pasture mixtures with perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) to create more diverse pasture mixtures. In comparison with standard perennial ryegrass-white clover pastures these mixtures have shown consistent reductions in urinary N concentration of at least 20% while maintaining or improving milk production (Woodward et al. 2012; Totty et al. 2013). However, it is not clear whether the effects are due to an individual species in the mixture rather than the diversity. There is currently little research on plantain as a single alternative species to perennial ryegrass species to achieve the same benefits seen in diverse pasture experiments.

The objective of this experiment was to compare milk production and urinary N concentration of late-lactation

dairy cows offered perennial ryegrass-white clover and pure plantain pastures in autumn.

### Materials and methods

#### *Experimental site and treatments*

The experiment was conducted at the Lincoln University Research Dairy Farm (LURDF) with the approval of the Lincoln University Animal Ethics Committee (AEC 592). A group of 36 pregnant, lactating Friesian x Jersey dairy cows were blocked according to milksolids production ( $1.31 \pm 0.1$  kg MS/cow/d), age ( $5.9 \pm 0.4$  years), days in milk ( $217 \pm 3.2$  days) and live weight ( $515 \pm 8.6$  kg) (all mean  $\pm$  sem). Animals were assigned from within blocks to graze one of three pasture treatments: (1) a perennial ryegrass-white clover pasture (n =12 cows); (2) plantain (n =12 cows) and (3) an area that was comprised of 50% perennial ryegrass-white clover and 50% pure plantain by ground area (n=12 cows). Cows grazed pastures for a period of ten days from 19 to 29 March 2015. Prior to the experiment, all cows rotationally grazed perennial ryegrass-white clover pasture together.

The perennial ryegrass-white clover pastures were established in March 2014 and the plantain pastures in December 2014. Pastures were grazed with dairy cows to constant height and fertilised with urea at a rate of 70 kg N/ha 25 days prior to the experiment. Pasture was irrigated with a centre-pivot irrigator. Cows were offered after each morning milking, a target daily allowance of 18 kg DM/cow/day above 1500 kg DM/ha post-grazing herbage. Cows were milked twice daily at approximately 0600 and 1400 h. Daily herbage allocations during the experiment were based on a national calibration equation for perennial ryegrass-white clover ( $\text{kg DM/ha} = 140 \times \text{RPM reading} + 150$ ) and previously derived calibration equations between herbage mass and pasture height for plantain pastures ( $\text{kg DM/ha} = 94 \times \text{RPM reading} + 455$ ) (Haultain et al. 2014). Daily allocated areas were controlled by temporary electric

fencing. Back fencing was used to prevent grazing of residual regrowth. Cows had *ad lib* access to water through a portable trough.

#### 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 expected to be allocated in the next forage allocation. Calibration measurements were collected from pastures every second day by cutting two 0.2 m<sup>2</sup> quadrats to ground level before and after grazing during the experiment. Two RPM measurements were recorded in each quadrat prior to harvesting herbage. Collected herbage samples were weighed fresh and a subsample taken to determine botanical composition of pastures. All botanical components and bulk samples were oven dried at 65°C for 48 h and total dry weight and DM% determined. Linear and curvilinear relationships between herbage mass and pasture height were compared and best-fit equations (greatest r<sup>2</sup>) were fitted to the data. The calibration equations for each pasture type were: perennial ryegrass-white clover (kg DM/ha) = 145 × height + 3.92, r<sup>2</sup> = 0.85, P>0.001, and plantain (kg DM/ha) = 73 × height + 248, r<sup>2</sup> = 0.89, P>0.001. Using equations derived from experimental data set and grazing areas, the actual daily herbage allocation was calculated as 21.4 ± 2.4 kg/DM/cow for pasture, 19.1 ± 0.94 kg/DM/cow for plantain and 22.2 ± 0.9 kg/DM/cow for 50-50 pasture-plantain. Apparent group DM intake of cows was calculated from herbage disappearance between pre- and post-grazing calibrated RPM measurements and areas allocated.

Subsampled herbage was sorted into sown grass, white clover, weeds and dead material for perennial ryegrass-white clover pastures and plantain, weeds and dead material for plantain pastures. Sorted material and a bulk sample of the pasture was oven dried at 65°C for 48 h and dry weight recorded to determine DM/ha and botanical composition of pastures. Bulk dried samples were 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). Metabolisable energy (ME) was calculated as MJ ME/kg DM = 0.16 × DOMD (CSIRO 2007).

#### Animal measurements

Milk yield was measured daily for individual cows with an automated system (Alpro Herd management system, Tumba Sweden). Two milk samples were collected every second day for every cow in the afternoon and following morning milking

to determine milk composition and milk urea nitrogen (MUN). The sample for milk composition was analysed by Livestock Improvement Corporation Ltd (Christchurch, New Zealand) to determine milk fat, protein and lactose by MilkoScan (Foss Electric, Hillerod, Denmark). The second sample for MUN was centrifuged at 4,000 × g for 10 min at room temperature and refrigerated for 10 min to allow the fat to solidify on the top and be removed. Skim milk was pipetted into a clean micro-centrifuge tube and frozen before analysis. Skim milk was analysed by Lincoln University Analytical Services for MUN by automated Modular P analyser (Roche/Hitachi).

Spot samples of urine and faeces were collected on days 4, 6 and 8 from all cows following morning and afternoon milking. Urine and faecal samples were analysed for N% and DM% as described by Miller et al. (2012).

#### Statistical analysis

The effect of pasture type on milk, urine and faecal measurements was analysed by ANOVA (GenStat 15.1 VSN International LTD. 2012), with cows as random effect and pasture type as fixed effect. For milk, urine and faecal measurements, 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 for plantain and ryegrass-white clover pastures are shown in Table 1. Post-grazing herbage mass was lower for plantain than ryegrass pastures (Table 1). ME and CP was similar across pasture treatments (range of 10.9 to 11.4 and 21.5 to 23.3, respectively). Water soluble carbohydrate (WSC) concentration was in higher in plantain than pasture.

**Table 1** Mean herbage characteristics (± sem) and chemical composition of pasture, plantain or 50-50 pasture-plantain sampled to ground level.

	Pasture	Plantain	50-50 pasture-plantain	
			Pasture	Plantain
<b>Pre-grazing</b>				
Herbage mass (kg DM/ha)	3209 ± 30.5	3187 ± 43.6	3935 ± 151	3005 ± 150
N (%)	3.7 ± 1.8	3.6 ± 0.3	3.4 ± 1.0	3.7 ± 0.9
ME (MJ ME/kg DM)	11.2 ± 0.1	11.4 ± 0.2	10.9 ± 0.1	11.3 ± 0.1
ADF (%)	28.2 ± 1.0	22.4 ± 1.3	29.5 ± 0.4	21.0 ± 0.3
WSC (%)	7.6 ± 0.7	10.9 ± 1.6	5.3 ± 1.3	12.6 ± 0.7
NDF (%)	42.9 ± 2.2	29.9 ± 3.0	49.1 ± 1.3	27.1 ± 0.7
CP (%)	23.3 ± 0.9	22.3 ± 0.6	21.5 ± 0.6	22.9 ± 0.5
Plantain (%)	0 ± 0	89.6 ± 2.5	0 ± 0	89.7 ± 2.1
Grass (%)	48.4 ± 5.0	0 ± 0	58.8 ± 4.0	0 ± 0
Legume (%)	25.7 ± 3.0	0 ± 0	15.1 ± 3.2	0 ± 0
Weed (%)	3.8 ± 3.0	6.1 ± 1.8	0.7 ± 0.3	3.7 ± 1.0
Dead (%)	22.1 ± 3.8	4.3 ± 1.1	25.4 ± 4.3	6.6 ± 2.2
DM (%)	17.1 ± 1.1	9.8 ± 0.3	16.8 ± 1.1	10.7 ± 0.4
<b>Post-grazing</b>				
Herbage mass (kg DM/ha)	1187 ± 11.3	611 ± 22.7	1024 ± 28.6	638 ± 18.4

**Table 2** Mean milk yield and composition of dairy cows grazing perennial ryegrass-white clover pasture, plantain or 50-50 pasture-plantain (n=12). LSD = least significant difference ( $\alpha = 0.05$ ). Means followed by different letters denote that values are significantly different at the 5% level

	Pasture	Plantain	50-50 pasture-plantain	LSD	P value
Milk volume (L)	14.3 <sup>a</sup>	16.4 <sup>b</sup>	16.3 <sup>b</sup>	0.76	<0.001
Milk solids (kg/d)	1.50 <sup>a</sup>	1.67 <sup>b</sup>	1.60 <sup>ab</sup>	0.08	0.012
Milk protein (%)	4.28	4.34	4.29	0.09	0.512
Milk protein (kg)	0.62 <sup>a</sup>	0.72 <sup>b</sup>	0.70 <sup>b</sup>	0.03	<0.001
Milk fat (%)	6.16 <sup>a</sup>	5.80 <sup>ab</sup>	5.52 <sup>b</sup>	0.23	<0.001
Milk fat (kg)	0.88	0.95	0.90	0.05	0.142
Lactose (%)	4.95 <sup>a</sup>	5.05 <sup>b</sup>	5.07 <sup>b</sup>	0.04	<0.001
Urea N (mmol/L)	11.2 <sup>a</sup>	9.96 <sup>b</sup>	10.9 <sup>a</sup>	0.54	0.005

**Table 3** Mean urine and faecal characteristics of dairy cows grazing perennial ryegrass-white clover pasture, plantain or 50-50 pasture-plantain (n=12). LSD = least significant difference ( $\alpha = 0.05$ ). Means followed by different letters denote that values are significantly different at the 5% level.

	Pasture	Plantain	50-50 pasture-plantain	LSD	P value
<b>Urine</b>					
NH <sub>3</sub> (mmol/L)	1.98 <sup>a</sup>	0.97 <sup>c</sup>	1.35 <sup>b</sup>	0.30	<0.001
Urea (mmol/L)	144.3 <sup>a</sup>	61.5 <sup>c</sup>	94.8 <sup>b</sup>	15.8	<0.001
N concentration (g N/L)	5.4 <sup>a</sup>	2.4 <sup>c</sup>	3.6 <sup>b</sup>	0.06	<0.001
Creatinine (mmol/L)	1.67 <sup>a</sup>	0.88 <sup>c</sup>	1.27 <sup>b</sup>	0.24	<0.001
<b>Faeces</b>					
N (%)	3.43	3.45	3.33	0.11	0.070
DM (%)	10.9 <sup>a</sup>	15.7 <sup>c</sup>	12.6 <sup>b</sup>	0.86	<0.001

Apparent DMI per daily grazing area was  $13.7 \pm 0.4$  kg DM/cow for pasture,  $15.9 \pm 0.5$  kg DM/cow/d for plantain and  $16.9 \pm 0.5$  kg DM/cow/d for 50-50 pasture-plantain ( $9.3 \pm 0.2$  kg DM/cow from perennial ryegrass pasture and  $7.6 \pm 0.2$  kg DM/cow from plantain). Apparent N intake was  $604 \pm 57.8$  g N/cow/day for pasture,  $593 \pm 23.5$  g N/cow/day for plantain and  $584 \pm 32.6$  g N/cow/day for 50-50 pasture-plantain.

#### Animal

Milk volume (L) was higher for plantain than pasture (Table 2). Milksolids yield was greatest for plantain intermediate for 50-50 pasture-plantain and lowest for cows grazing pasture (Table 2). There was no significant difference in the milk protein percent among treatments (Table 2). Milk fat percent was lower for 50-50 pasture-plantain than for pasture (Table 2). Cows grazing plantain and 50-50 pasture-plantain produced more milk protein than those grazing pasture. Milk urea N was lowest for plantain. The concentration of NH<sub>3</sub>, urea and N in urine was lowest for plantain, intermediate for 50-50 pasture-plantain and highest in pasture (Table 3). The concentration of N in the faeces was similar among treatments.

## Discussion

Milksolid production per cow was greatest for cows grazing plantain (1.67 kg) compared to pasture (1.50 kg) with 50-50 pasture-plantain intermediate (1.6 kg). This was largely due to an increase in milk volume, by cows grazing

plantain or 50-50 pasture-plantain. Milk protein percentage was unaffected by pasture treatment and fat percentage tended to be lower where plantain was included in the diet. A possible explanation for the increase in milk volume was the increase in apparent DMI for cows on plantain. This occurred because plantain was grazed to a lower post-grazing herbage mass compared with pasture despite, similar daily allocations of pasture. However, it is noteworthy that cows grazing 50-50 pasture-plantain had the highest apparent DMI and this did not translate to increased milk production.

Previous short term experiments have shown that when plantain is included in mixtures with ryegrass, white clover and chicory, urinary N concentration of dairy cows were reduced by at least 20% compared with perennial ryegrass-white clover pastures (Woodward et al. 2012; Totty et al. 2013). In the current experiment, urinary

N concentration was 50% and 33% lower, respectively for plantain and 50-50 pasture-plantain, compared with pasture. The MUN concentration, an indicator of surplus dietary N (Cosgrove et al. 2014), was also lower in plantain than pasture or 50-50 pasture-plantain. Previous studies have shown the excretion of N in urine to be linearly related to N intake (Tas et al. 2006; Higgs et al. 2012). In this experiment the difference in apparent N intake between pasture and plantain was small at 11 g N/cow/day, and is unlikely to be sufficient to explain the large (3 g N/L) difference in urine N concentration. It is possible that the higher WSC concentration of plantain compared to pasture (10.9 vs 7.61 g/kg DM) contributed to the lower urine N concentration of urine from cows with plantain (Edwards et al. 2007), although values were low (>10%) and are unlikely to explain differences in high CP (>21%) forages seen in this study. Another explanation for lower urine N concentration is a greater urine volume from cows grazing plantain as a consequence of with higher mineral content (Ledgard et al. 2015), secondary plant compounds or increased water intake due to a lower DM% of plantain. Some indication of this is the 47% decrease in creatinine, a marker of urine volume (Chizzotti et al. 2008; Waldrip et al. 2013), for cows grazing plantain compared with pasture.

## Conclusions

The results demonstrate a role for the use plantain as a mitigation strategy to reduce the environmental impact of dairy farming. By providing plantain as a monoculture

or with perennial ryegrass-white clover pastures to cows in late lactation, milksolids production was increased and urine N concentration was reduced. The decline in urine N concentration may decrease N loading from the urine patch and reduce the risk of nitrate leaching for dairy grazing systems.

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