

## **PLANTAIN (*Plantago lanceolata*) NITROGEN USE AND EXCRETION BY LACTATING DAIRY COWS**

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### **Abstract**

The incorporation of plantain (*Plantago lanceolata*) in cows' diets can reduce the urinary N concentration (UNc) and potentially reduce dairying's environmental footprint. However, to provide farmers with confidence in using plantain-based swards, research needs to demonstrate that these environmental benefits are not at the expense of milk production and farm profit. This research examined the effect of grazing plantain on milk production and urine-N excretion by cows in an experiment conducted at Massey University's Dairy 4 Farm throughout two lactation seasons (2017-2018 and 2018-2019). Three mobs of 20 cows were matched for age, weight and milk production, and assigned to graze three pasture treatments: (i) plantain, (ii) plantain-clovers mix, (plantain, red [*Trifolium pratense*] and white clover [*T. repens*]), or (iii) ryegrass (*Lolium perenne*)-white clover (wc). The pastures were established (1 December 2016) in a complete randomised design with five replicate plots (800 m<sup>2</sup>) for each treatment that were grazed from spring (September) to autumn (May). Cows were acclimatised to each pasture treatment for 6 days (adaptation period) before grazing the experimental plots (4 cows/plot) for 2 days (experimental period). Pasture intake, diet quality, and animal N (milk, urine and faeces) were measured during the experimental period in spring (September 2017, December 2017/18), summer (February 2018/19), and autumn (March 2018, May 2019) for both lactations. The cows grazing the plantain and plantain-clover mix pastures produced the same quantity of milk solids ( $P>0.05$ ) as cows grazing ryegrass-wc pasture throughout both lactation seasons. Both plantain and the plantain-clover mix reduced ( $P<0.01$ ) the UNc by 36 and 40% in the summer and autumn 2017-18, respectively, when compared with ryegrass-wc. However, the UNc in cows grazing plantain was 10 and 21% lower ( $P<0.01$ ) during the summer and autumn 2018-19, respectively, when compared to those grazing the plantain-clover mix and ryegrass-wc pastures. The results demonstrate that plantain pastures do not diminish milk solids production from cows and the lower UNc from summer to autumn could reduce N being lost to the environment.

## **Introduction**

New Zealand pastoral dairy systems require management practises to reduce the nitrogen (N) loss from dairy farms, principally the nitrate ( $\text{NO}_3^-$ ) leaching into ground waters. The high N loading in cows' urine is the main N source to be lost as  $\text{NO}_3^-$  leaching in grazing systems (Di and Cameron, 2002). Strategies developed to reduce the  $\text{NO}_3^-$  leaching from pastoral systems involves reducing the urinary load while cows are at pasture, such as the feeding of supplements and crops with pasture diets (Kymanywa et al., 2020) or housing cows and/or controlling the grazing duration (Christensen et al., 2019). All options involve significant increases in the costs of feed supply and /or the infrastructure to house or feed supplements (Beukes et al., 2013). The lower urinary N concentration (UNc) of cows grazing pastures containing plantain (*Plantago lanceolata* L.) creates an opportunity to modify pastures and decrease the urinary load of grazing cows in a more cost effective manner (Totty et al., 2013; Bryant et al., 2016; Box et al., 2018). To provide farmers with confidence in using plantain-based swards, research needs to demonstrate that these environmental benefits are not at the expense of milk production and farm profit. This paper reports an evaluation of the effect of plantain pastures on the urinary N load from lactating cows and the milk solids (MS) production, in comparison with the traditional ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) based-pasture throughout two lactating seasons.

## **Materials and Methods**

### ***Experimental design and treatments***

A dairy system experiment was conducted at the Massey University Dairy Farm No 4 in Palmerston North, New Zealand (40° 23' S; 175 ° 36' E) with the approval of the Massey University Animal Ethics Committee (Protocol 16/137). The experiment evaluated three pasture-based diets (treatments) sown on 1 December 2016, which were: (i) plantain, (ii) plantain-clovers mix (containing plantain, red clover and white clover), and (iii) perennial ryegrass-wc pastures. The pasture treatments were established in a complete randomised design (CRD) with five replicate plots (40 x 20 m), and the area surrounding the experimental plots was divided into three outside areas (approximately 1.0 ha each). These three outside areas were sown to each pasture treatment and in each grazing rotation were used to adapt the cows' diet to the pasture treatments before placing the cows in the experimental plot treatments.

### ***Grazing management***

Sixty multiparous lactating cows (Friesian x Jersey) were selected from the Dairy Farm No 4 herd and separated in three groups (n=20 cows/pasture). Each group of cows grazed one of the three pasture treatments – approximately 8-9 days every month – throughout two full lactation season (i) from September 2017 to June 2018 (First season) and (ii) from September 2018 to May 2019 (Second season). Cows grazed first in the outside area for 6 days before spending 2-3 days in the experimental plots (4 cows/plot). Cows were transitioned from the herd diet to the pasture treatments over a 3-day period. During these transition days cows grazed in a ryegrass-wc pasture after the morning (AM) milking and in their pasture treatments after the afternoon

(PM) milking. During every grazing cows were supplemented with varying quantities (5-8 kg DM/cow/day) of supplements, but typically included maize silage, dairy pellet, dried distiller grains (DDG) and baleage, peas, and soy hull. Once the grazing period was completed all cows returned to the farm herd until the next grazing.

### ***Animal Measurements***

Milk yield (L/day) was recorded daily for each cow with an automated system and milk samples (35 mL) were collected from each cow during the morning (AM) and afternoon (PM) milking on day 1 (D1) and day 8 (D8) to determine milk solids (MS) production (fat + protein). Milk solids production was evaluated in spring (September 2017 and 2018), early summer (December 2017 and December 2018), late summer (February 2018 and 2019), and autumn (March 2018 and May 2019) throughout two lactating seasons (2017/2018 and 2018/2019).

Spot urine samples were collected from each cow by manual stimulation of the vulva on day 7 (D7) and D8 immediately after the AM milking. Urine samples were collected in spring (September 2017), early (December 2017) and late summer (February 2018), and autumn (March 2018) during the 2017/2018 season. During the 2018/2019 seasons, urine samples were collected in early summer (December 2018), late summer (February 2019), and autumn (May 2019). One set of urine samples was acidified with sulphuric acid (6N H<sub>2</sub>SO<sub>4</sub>) to reduce the urine pH to 3.0 - 4.0 and the other set was prepared with normal urine (non-acidified) to later analyse for N and creatinine concentration, respectively. Urinary N excretion (g N/day) and urine volume (L/day) were estimated from the equation described by Pacheco et al. (2009).

### ***Pasture treatments measurements***

Herbage mass (kg DM/ha) pre- and post-grazing and the botanical composition for each pasture treatment were determined in each season. Three pre- and three post-grazing herbage samples (quadrats 0.1 m<sup>2</sup>) were randomly taken from each plot by cutting pasture at ground level with an electric shearing handpiece. All herbage samples were washed to remove soil contamination and oven dried at 70 °C for approximately 48 h. The botanical composition and nutritive value of the pastures were evaluated four times (season) during the season 2017/2018: (i) September (spring), (ii) December (early summer), (iii) February (late summer) and (iv) March 2018 (autumn). For botanical composition, three herbage samples were taken from each plot by cutting the herbage (quadrats 0.1 m<sup>2</sup>) at ground level using an electric shearing handpiece. Each botanical sample was manually separated into each pasture species (ryegrass, plantain, white clover, red clover, weeds) and dead material, and oven-dried individually at 70 °C (approximately 48 h). The proportion for each species in the total dry matter (DM) intake was then calculated. One grab sample (200 g fresh weight) from each plot was taken pre-grazing for nutritive analysis.

### *Statistical analysis*

Data were analysed using the PROC MIXED procedure of SAS 9.3 (SAS Institute, 2009) using the model for a complete randomise design. Means were compared using the least squares means test and significance was declared at  $P < 0.05$ .

## **Results**

### *Milk production*

The MS production (kg MS/cow) from cows after 8 days grazing in the pasture treatments was similar between plantain, plantain-clovers mix, and ryegrass-wc pastures in all seasons evaluated (Table 1). The herbage harvested by dairy cows in the 2017/2018 lactating season was similar for the ryegrass-wc ( $11.8 \pm 0.58$  t DM/ha) and plantain ( $10.6 \pm 0.63$  t DM/ha) pastures, but 16% more herbage was harvested when cows grazed in the plantain-clovers mix pasture ( $13.4 \pm 1.28$  t DM/ha). However, in the 2018/2019 lactating season, cows grazing plantain ( $8.3 \pm 0.27$  t DM/ha) pastures harvested 19% less herbage than cows grazing in the ryegrass-wc ( $10.3 \pm 0.81$  t DM/ha) or plantain-clovers mix pastures ( $10.2 \pm 0.91$  t DM/ha).

**Table 1.** Mean daily milk solids production (kg MS/cow) from lactating cows grazing ryegrass-wc, plantain, plantain-clovers mix pastures across the 2017/2018 and 2018/2019 seasons

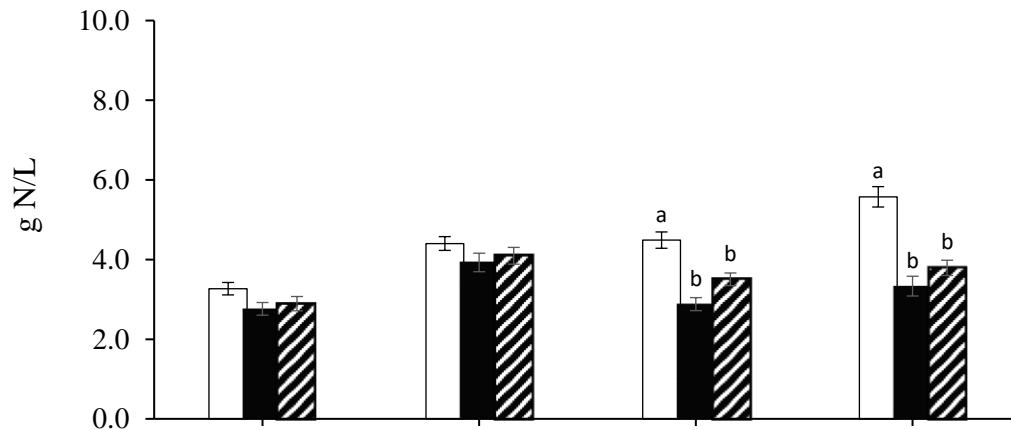
	<b>Ryegrass-wc</b>	<b>Plantain</b>	<b>Plantain-clovers</b>	<b>P-value</b>
<b>Season 2017-2018</b>				
Spring	1.50	1.43	1.60	0.33
Early summer	1.73	1.47	1.31	0.17
Late summer	1.35	1.40	1.45	0.75
Autumn	1.27	1.16	1.19	0.26
<b>Season 2018-2019</b>				
Spring	2.44	2.32	2.44	0.78
Early summer	1.86	2.07	2.01	0.67
Late summer	1.34	1.44	1.46	0.30
Autumn	1.01	1.10	1.06	0.71

### *Nitrogen excretion*

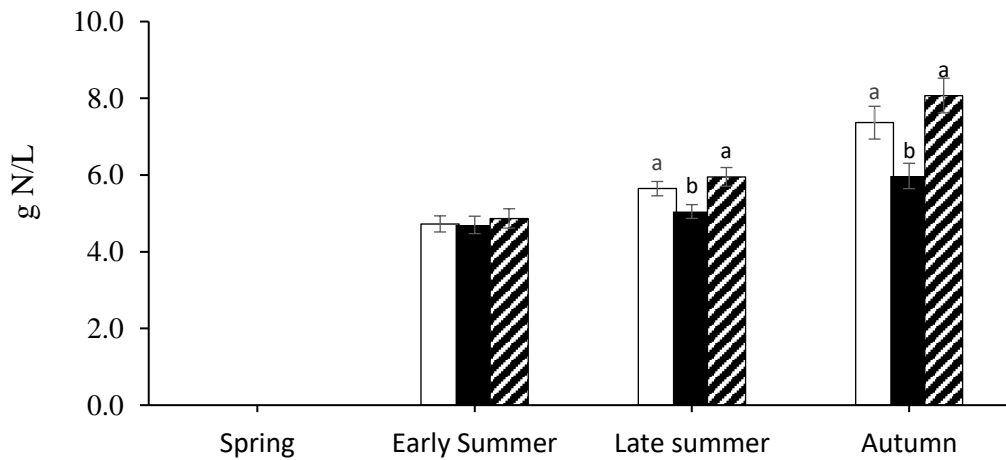
The N concentration in the urine of cows grazing plantain, plantain-clovers mix, and ryegrass-wc pastures in both 2017/2018 and 2018/2019 seasons is presented in Figure 1. During spring and early summer, the UNc for cows grazing plantain and plantain-clovers mix were similar to those grazing ryegrass-wc pastures in the 2017/2018 and 2018/2019 lactating seasons. Both plantain and plantain-clover mix pastures significantly reduced ( $P < 0.01$ ) the UNc of cows in late summer to autumn in comparison with ryegrass-wc pastures (Figure 1). During the 2017/2018 season, the UNc was on average 29 and 36% lower in late summer and autumn,

respectively in cows grazing both plantain and plantain-clovers mix pastures than ryegrass-wc pasture. In the 2018/2019 season, cows grazing plantain pasture had a UNc 13 and 23% lower in late summer and autumn, respectively, compared with cows grazing ryegrass-wc and plantain-clover mix pastures.

### A) Season 2017-2018

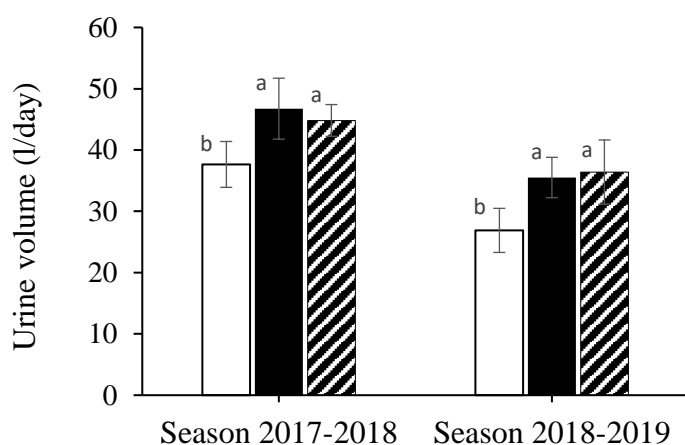


### B) Season 2018-2019



**Figure 1.** Mean urine N concentration (g N/L) on cows grazing in ryegrass-wc (white), plantain (black), plantain-clovers mix (diagonal lines) pastures treatments across the A) 2017/2018 and B) 2018/2019 seasons.

The urine production (l/day) estimated for cows grazing in the three pasture treatments showed that cows grazing both plantain and plantain-clovers mix pastures produced 22 and 24% more urine both in the 2017/2018 and 2018/2019 seasons, respectively (Figure 2).



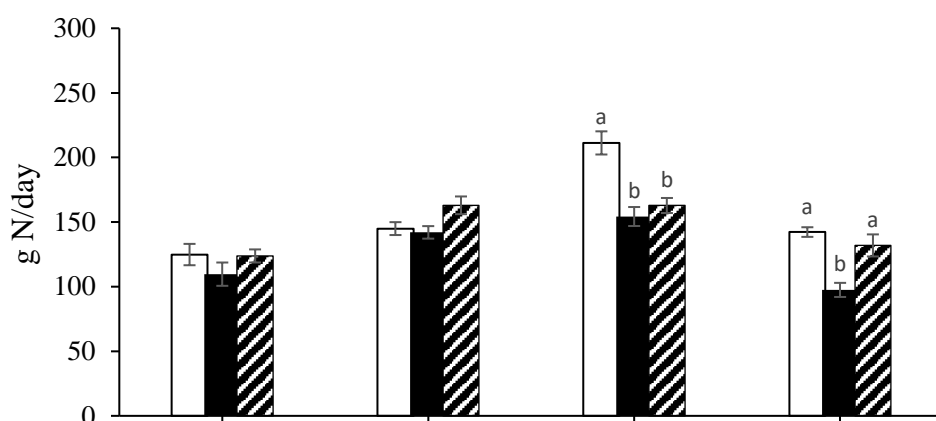
**Figure 2.** Mean urine volume (L/day) estimated for cows grazing in the ryegrass-wc (white), plantain (black), plantain-clovers mix (diagonal lines) pastures treatments during the 2017/2018 and 2018/2019 lactating seasons.

The total N excretion (g N/day) estimated in the urine of cows grazing plantain, plantain-clovers mix, and ryegrass-wc pastures in the 2017/2018 and 2018/2019 season are presented in Figure 3. During the 2017/2018 lactating season, the total N excreted by lactating cows grazing plantain and ryegrass-wc pastures in spring and early summer was similar, but 27 and 31% less N was excreted in the urine of cows grazing plantain than ryegrass-wc pasture. While in the 2018/2019 lactating season, cows grazing plantain and plantain-clovers mix pastures excreted more N in the urine than cows grazing ryegrass-wc (Figure 3).

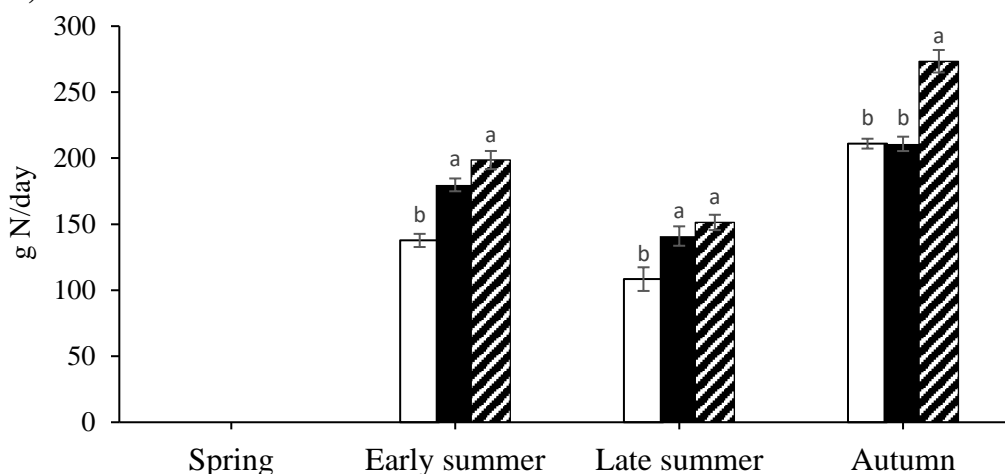
## Discussion

Plantain is a potential forage option to help NZ farmers to mitigate nitrate-N leaching to the environment from dairy farming. In this research, changing cows' diet to pure plantain, or plantain-clovers mix pastures, sustained the same MS production when compared with the traditional ryegrass-wc pasture diet across two lactating seasons. At the same time, greater urine volumes were estimated for cows grazing both plantain and plantain-clover mix base-pastures. The N concentration in the urine of cows grazing plantain or plantain-clovers mix was reduced in late summer and autumn when compared with the urine of cows grazing ryegrass-wc pasture. Since the major source of  $\text{NO}_3^-$  leaching from grazed pastures came from the N excreted in the urine of cows, reducing both the concentration of N in urine (loading rate) and/or the total N loading into the soil would lead to reduced risk of nitrate-N leaching from pastoral systems (Li et al., 2012; Ledgard et al., 2015).

### A) Season 2017-2018



### B) Season 2018-2019



**Figure 3.** Mean total N excretion (g N/day) in the urine of cows grazing ryegrass-wc (white), plantain (black), plantain-clovers mix (diagonal lines) pastures treatments in the A) 2017/2018 and B) 2018/2019 lactating season.

Despite N intake being the biggest factor influencing the amount of N excreted in cows' urine, the decline in the UNc of cows grazing plantain could be a consequence of dietary factors leading to increased urine production (Pacheco et al., 2010). The lower DM content and higher mineral concentration in plantain indicated that the lower UNc for cows grazing plantain pastures was, in part, via a dilution effect as cows produced greater urine volumes compared with cows grazing ryegrass-wc pasture (Beukes et al., 2014). The lack of differences in the UNc on cows grazing plantain or ryegrass-wc pastures observed in spring and early summer also suggested that other plantain traits could be influencing the N excreted in cows' urine and subsequent  $\text{NO}_3^-$  leaching from plantain-based pastures. For example, slower CP degradation of plantain in the rumen may be contributing to reduce the UNc from late summer to autumn (Minnee et al., 2017). In this research, the higher WSC concentration in the herbage of plantain pastures compared to ryegrass-wc, and combined with more plantain in the diet of cows during

the 2017/2018 season (55% plantain) compared with the 2018/2019 season (22% plantain) explained the lower total N excreted in the urine of cows grazing plantain pastures in the first year of evaluation. The presence of bioactive secondary compounds (aucubin and acteoside) in plantain could also play a role on the cows nutrients utilisation, and thus the N excretion by lactating cows (Navarrete et al., 2016). In summary, the power of plantain to reduce the NO<sub>3</sub><sup>-</sup> leaching from dairy pastoral systems could result from less urine N loading into the soil caused by the combined effect of lower partitioning of N into urine and a more diluted cows' urine.

## Conclusions

Plantain pastures can be included in cows' diets to create lower urinary N without loss in milk production to provide a pasture option with the value to reduce the NO<sub>3</sub><sup>-</sup> leaching from NZ pastoral dairy systems.

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