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## **EFFECT OF PLANTAIN CONTENT IN RYEGRASS-BASED DAIRY PASTURES ON NITRATE LEACHING AND KEY COMPONENTS OF THE NITROGEN CYCLE**

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### **Abstract**

The objective of this study was to quantify the effect of plantain inclusion and plantain content in ryegrass-based pastures on nitrate leaching and key components of the nitrogen (N) cycle of a pastoral dairy system. Four pasture treatments: perennial ryegrass-white clover (RGWC), RGWC + low plantain rate (L), RGWC + medium plantain (M), and RGWC + high plantain (H) were established in a randomized complete block design with five replicate plots of 800 m<sup>2</sup> each. The measurements of nitrate leaching, urinary N (UN) excretion, faecal N excretion, clover-fixed N, and N uptake by pastures were conducted over two drainage seasons from January 2020 to December 2021. During this period, the pastures were rotationally grazed with lactating cows under normal grazing management with nine grazing rotations per year. Nitrate leaching was estimated from drainage volume and N concentration in drainage water that were recorded and collected from an isolated drainage system set up in each plot. Nitrogen excretion from cow urine and dung was estimated from N concentration in urine and faecal spot samples. Clover-fixed N and N uptake by pastures were estimated from clover content in the sward, herbage dry matter (DM) yield, and N content in the herbage. The results showed that the inclusion of plantain into pastures reduced nitrate leaching by 40% compared to the standard RGWC pasture. This reduction in N leaching was greater with an increase in plantain content, and resulted from decreased UN excretion and increased N uptake by pastures.

### **1. Introduction**

Nitrogen (N) loss, especially nitrate leaching from pastoral dairy systems into waterways, is a significant concern in New Zealand and is limited by strict regulations (Minister for the Environment, 2020). Incorporating plantain into the standard perennial ryegrass-white clover pasture (RGWC) has been suggested to reduce the N concentration in cow urine and subsequently reduce urinary N (UN) excretion (Nguyen et al., 2022), inhibit denitrification processes in the soil (Podolyan et al., 2019), and therefore, increase the amount of N uptake by pastures and reduce nitrate leaching from the pastoral systems (Carlton et al., 2019). However, there have been no studies comprehensively measuring the effect of plantain content of pastures on different components of the N cycle in pastoral dairy systems.

The aim of the current study was to quantify the effect of plantain inclusion and plantain content in ryegrass-based pastures on nitrate leaching and key N components of the N cycle of a

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pastoral dairy system. Our hypothesis was that an increase in the plantain content in grazing pastures reduces N leaching from the pastoral systems. This reduction in N leaching is associated with a decrease in UN excretion and an increase of N uptake by pastures. The result of the current study was expected to provide a better understanding of the effect of plantain content on nitrate leaching in association with key components of the N cycle and confirm suitable rates of plantain in ryegrass-based pastures.

## **2. Materials and Methods**

### ***2.1. Experimental site, treatment, and management***

The study was conducted at the Massey University Dairy Farm No 4 in Palmerston North, New Zealand (40° 23' 30" S, 175° 36' 45" E) with the approval of the Massey University Animal Ethics Committee (19/54). The experimental design was a randomized complete block design with four pasture treatments and five replicates. The treatments were four mixed swards sown with increased plantain seed rates versus RGWC. The treatments were: RGWC, RGWC + 4 kg plantain (L), RGWC + 7 kg plantain (M), and RGWC + 10 kg plantain (H).

Pastures were sown on April 5<sup>th</sup>, 2019, with 20 experimental plots (20 m x 40 m each) and four adaptation areas (1.0 ha each). The pastures were rotationally grazed by dairy cows with nine grazing events per year over the study period from September 2019 to December 2021. In each grazing event, cows first grazed for 6 days in the adaptation areas before moving to graze 2-4 days in the experimental plots. Cows were managed under normal farm conditions and offered at least 14 kg dry matter (DM)/cow/day of pasture diet and between 5 – 10 kg DM/cow/day of supplements. Pastures were fertilised with a total of 100 kg N/ha/year of urea and mowed four times during the study period for weed and seed head control.

### ***2.2. Measurements***

#### ***2.2.1. Pasture measurement***

Pastures were measured to estimate herbage DM production (kg DM/ha), botanical composition and nutritive value in each grazing event. To measure herbage DM production, three samples were randomly cut to ground level (0.1 m<sup>2</sup> quadrat) from each plot before and after grazing and oven-dried at 75°C until a constant weight was achieved. Herbage DM production was estimated by deducting the pre-grazing DM mass at the subsequent grazing from the post-grazing DM mass at the previous grazing. To measure the botanical composition and nutritive value, a hand-plucked sample comprising 15-20 random grabs from each plot was mixed thoroughly and divided into two sub-samples for botanical composition separation and nutritive value analysis. The botanical composition sub-samples were manually separated into perennial ryegrass, white clover, plantain leaves, plantain reproductive stem and seed head, weeds, and dead material, and oven-dried at 75°C until a constant weight was achieved to calculate the composition of each pasture species. The nutritive value sub-samples were weighed to record the fresh weight and then oven-dried at 60°C until a constant weight was

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achieved, then ground through a 1-mm sieve. Ground samples were analysed for nutritive value N content, metabolisable energy (ME), neutral detergent fibre (NDF) by a commercial laboratory using near infra-red (NIR) spectroscopy

### 2.2.2. *Animal measurement*

The N excretion in urine and dung was measured in the late lactation period over seven grazing events: December 2019, February 2020, March 2020, December 2020, February 2021, March 2021, and May 2021. Urine and faecal samples were collected from each cow after morning milking on day 7 and day 8 of the grazing period when cows were grazing in experimental plots. Urine samples were collected by vulva stimulation, then acidified to reduce pH to below 3.0-4.0 to analyse UN concentration and creatinine. Faecal samples were taken by rectal stimulation, freeze-dried and ground to analyse faecal N concentration.

### 2.2.3. *Nitrate leaching and total N loss measurement*

Nitrate leaching and total N loss were measured for individual plots after each drainage event. An isolated mole and pipe drainage system was established for each experimental plot before the pastures were sown. Drainage volume was recorded by a flow meter and used to calculate the total drainage volume leaching from each plot. A drainage water sample was collected after each drainage event from a container attached to drainage system. The sample was divided into two sub-samples. One was filtered through a 0.45 µm filter for the analysis of nitrate N concentration. The other was digested in an autoclave at 120°C for one hour for the analysis of total N concentration.

## 2.3. *Statistical calculation and analysis*

### 2.3.1. *Calculation*

The contributions of N components of the N cycle were quantified using the measured data and available equations from previous studies. Key components of the N cycle included excreted N in urine, excreted N in dung, clover-fixed N as the inputs, and N uptake by pastures and N losses in drainage water as the outputs (Smit et al., 2021). Detailed calculations are described below:

- (a) Urinary N (kg/ha/year) =  $UN_{\text{excretion}} \text{ (g/cow/day)} \times \text{grazing period (cow.day/ha/year)}$ ;  
where  $UN_{\text{excretion}} \text{ (g/cow/day)} = 21.9 \times BW \text{ (kg)} \times (1/\text{urinary creatinine (mg/kg)}) \times \text{urine N (g/kg)}$  (Pacheco et al., 2007)). Where BW is body weight.
- (b) Faecal N (kg/ha/year) =  $\text{faecal N}_{\text{excretion}} \text{ (g/day/cow)} \times \text{grazing period (cow.day/ha/year)}$ ; where  $\text{faecal N}_{\text{excretion}} \text{ (g/cow/day)} = [72.7 - 11.8 \times ME - 0.437 \times NDF + 3.52 \times CP + 0.161 \times \text{forage rate (\%)} + 9.32 \times DMI - 0.0184 \times DIM]$  (Reed et al., 2015)) + plantain effect; in which plantain effect =  $\text{faecal N}_{\text{excretion}} \times 0.0016 \times \text{plantain content (\%)}$  (Minnée et al., 2020b). Where ME is metabolisable energy, NDF is neutral detergent fibre, CP is crude protein, DMI is dry matter intake, and DIM is day in milk.

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(c) Clover-fixed N (kg/ha/year) =  $28 \times \text{clover content (\%)} \times \text{pasture yield (t DM/ha)}$  (Lucas et al., 2010).

(d) Nitrate leaching (kg nitrate-N/ha/year) =  $\text{drainage volume (mm)} \times \text{nitrate-N concentration (g/L)}$ .

(e) N uptake (kg N/ha) =  $\text{N pasture (\%DM)} \times \text{pasture yield (kg DM/ha)}$ .

### 2.3.2. Analysis

Data were analysed using PROC GLM procedure of SAS 9.4 (SAS Institute, 2020), with the fixed effect of pasture treatment. Means were compared using Fisher's least square difference. Significance was declared at  $P < 0.05$ .

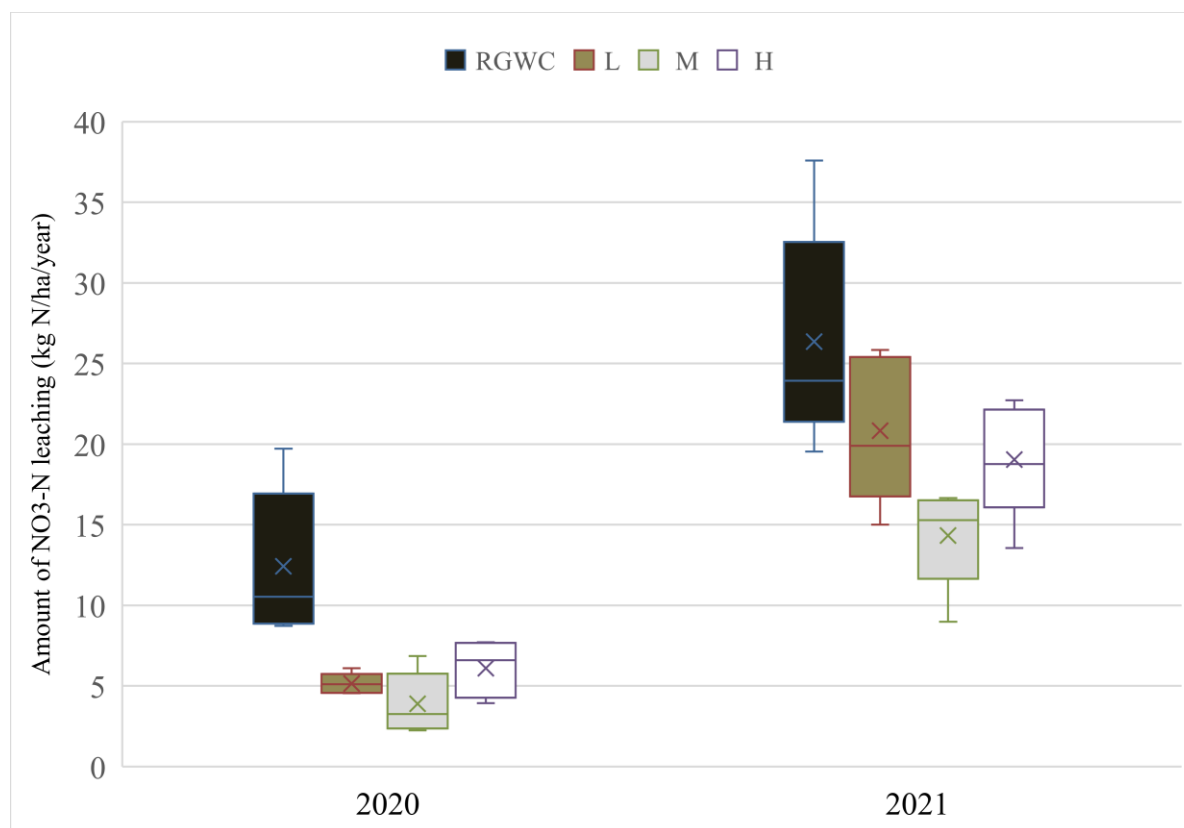
## 3. Results

Herbage DM yield in the experimental period was 14,600 kg DM/ha/year. The average percentage of plantain was 31%, 44% and 48% in L, M, and H, respectively. A total of 16 drainage events occurred over the two-year period, with an average drainage volume of 101 mm in 2020 and 286 mm in 2021.

### 3.1. Nitrate leaching

Nitrate leaching from the pastoral systems in 2020 and 2021 is presented in Figure 1. The amount of N leaching across treatments in 2021 was nearly double that in 2020. Incorporation of plantain decreased N leaching from the pastoral systems in both years. Over the experimental period, the amount of N leaching from the pastures containing plantain was 40% lower than from the system with RGWC ( $P < 0.05$ ). Among treatments with plantain, M pastures tended to have a lower N leaching loss, however, no significant difference was found between L, M or H pastures ( $P > 0.05$ ).

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**Figure 1.** Nitrate leaching loss from the pastoral systems with perennial ryegrass (RGWC), low plantain-RGWC (L), medium plantain-RGWC (M), and high plantain-RGWC (H) in 2020 and 2021.

### 3.2. Key components of the nitrogen cycle

There were significant differences in UN excretion and faecal N excretion between pastoral systems ( $P < 0.05$ ). Overall, the pastoral systems containing plantain were 10% lower in UN excretion and 11% higher in faecal N excretion, compared with RGWC. The effect was significant on UN and faecal N excretion only with the M or H pastures, and not with the L pasture, compared with RGWC.

**Table 1.** Key components of the nitrogen (N) cycle in the pastoral systems with perennial ryegrass (RGWC), low plantain-RGWC (L), medium plantain-RGWC (M), and high plantain-RGWC (H) from January 2020 – December 2021.

N source	RGWC	L	M	H*	SEM	P-value
Nitrate leaching (kg N/ha/year)	19.4 <sup>a</sup>	13.0 <sup>b</sup>	9.1 <sup>c</sup>	12.6 <sup>bc</sup>	1.2	<0.001
Urinary N (kg N/ha/year)	152 <sup>a</sup>	148 <sup>ab</sup>	124 <sup>c</sup>	142 <sup>b</sup>	2.7	<0.001
Faecal N (kg N/ha/year)	114 <sup>a</sup>	122 <sup>ab</sup>	128 <sup>bc</sup>	130 <sup>c</sup>	3.1	0.003
Clover-fixed N (kg N/ha/year)	63	62	67	76	7.7	0.550
N uptake (kg N/ha/year)	445	466	481	434	15.1	0.137

\* In April 2021, plots of H treatment were sprayed to remove perennial ryegrass and direct drilled with plantain seed to increase plantain content. This intervention may influence N sources from April

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to December 2021.

In addition, no significant difference was found in clover-fixed N and N uptake by pastures ( $P > 0.05$ ), however, there was a clear trend showing that the pastures containing higher plantain rates had a higher amount of clover-fixed N and N uptake by pastures, except the H treatment which may be affected by the intervention in the second year (see the note under Table 1).

#### **4. Discussion**

The results of the current study confirm the hypothesis that incorporating plantain into a RGWC pasture reduces nitrate leaching. The effect of plantain in reducing the N leaching by 40% in this study was relatively high at the paddock scale, but much lower than the reduction of 82% in the study of Carlton et al. (2019), conducted using lysimeters with 700 kg N urine/ha applied. While the effect of plantain inclusion on reducing N leaching was consistent, various factors such as the level of urine application, soil type, and the farm system may influence the scale of this effect, and further studies are required to confirm this (Cameron et al., 2013; Carlton et al., 2019).

The effect of plantain in reducing N leaching was associated with a decrease in UN excretion, and possibly an increase in N uptake by pastures. In the current study, the total amount of UN excretion was 10% lower, while N uptake by pastures was 6.5% higher in the pastures containing plantain (except the H treatment). Plantain contains aucubin and has a high concentration of soluble carbohydrates that can reduce ammonium production in the rumen (Navarrete et al., 2016), and it increases N partitioning to milk and faeces, while reducing N to urine (Minnée et al., 2020a). In addition, plantain is 1.5 times higher in macro-mineral content than RGWC, and this might result in a higher urine volume and urinary frequency, which would dilute and spread UN onto a larger area, resulting in greater, or at least more even, uptake by pastures of UN. The amount of N uptake by pastures accounted for more than 90% of the output N in the current study. Therefore, the small increase (6.5%) in N uptake could potentially result in a large reduction in N leaching loss.

This study also indicated that the effect of plantain was greater with higher levels of plantain included in a pasture. The effect of plantain was only significant for reducing UN excretion in M pastures (44% plantain), or for increasing faecal N excretion in H pastures (48% plantain). However, a lower plantain content (31% in the L pastures) was sufficient to significantly reduce nitrate leaching. This was because the reduction in nitrate leaching was the accumulated result of several factors, including the decrease in UN excretion and the increase in N uptake by pastures.

#### **5. Conclusion**

Pastures containing plantain can reduce N leaching from a pastoral dairy system. The reduction in N leaching is greater with a higher plantain rate and is associated with a decrease of UN excretion and an increase of N uptake by pastures.

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