IMPACT OF PLANTAIN (*Plantago lanceolata*) BASED PASTURE ON MILK PRODUCTION OF DAIRY COWS AND NITRATE LEACHING FROM PASTORAL SYSTEMS

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ABSTRACT

In temperate dairy systems, the traditional perennial ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) (RGWC) pasture often has excessive nitrogen (N) content relative to the N requirement of animals, posing a risk of nitrate (NO_3^-) leaching into the environment. Recently, incorporating plantain (*Plantago lanceolata*) with RGWC pasture has been increasingly used to improve economic and environmental benefits for dairy farms. However, the impact of plantain incorporation on farm productivity and NO_3^- leaching at the farm level has not been fully understood. The objectives of this thesis were to quantify the effect of incorporating increasing rates of plantain in grazing swards on pasture production, milk yield and composition of dairy cows, and NO_3^- leaching from pastoral dairy systems.

To address the objectives of the thesis, a grazing trial was implemented at a research dairy farm between September 2019 and December 2021. Pasture treatments were RGWC (perennial ryegrass cv. ONE^{50} and white clover cv. Tribute), RGWC + low plantain (cv. Agritonic) rate (PLL), RGWC + medium plantain rate (PLM), and RGWC + high plantain rate (PLH). Pastures were established with 20 experimental plots and four adaptation areas in April 2019 and were rotationally grazed by dairy cows over 22 grazing events during the experimental period. In each grazing, 60 or 80 cows were assigned to graze for 6 days in their adaptation areas and 1.5–3 days in the experimental plots. The experimental cows were managed under a typical practice, milking twice daily, offering grazing pasture and approximately 25% supplementary dietary feeds. Measurements were conducted to quantify the yield, botanical composition and nutritive value of the pasture, milk yield, milk composition and N excretion of dairy cows, and NO_3^- leaching from the pastoral system.

The results showed that, over the first two lactation years after sowing, plantain-based pastures[1] have a similar dry matter yield and contain higher water content, non-structural carbohydrates, minerals, and bioactive compounds than the RGWC pasture. The average plantain proportion in the swards over the first two years after sowing was 32% in PLL, 44% in PLM, and 48% in PLH, which increased in the first 15 months and declined to 20% in PLL and 30% in PLM and PLH at day 705 after sowing. Cows grazing the plantain-based pastures had a similar milk yield, composition and yield of solids, protein, fat, and lactose as those grazing the RGWC pasture. Furthermore, when 25% plantain was included in the diet of cows in late lactation, it resulted in a 44% increase in urine volume and a 29% reduction in urine N concentration by 29%. By incorporating an average of 30% and 50% plantain with RGWC pasture, NO_3^- leaching was reduced by 32 and 52%, respectively, over two drainage years after establishment, with a greater reduction in the first year than in the second year. Among sowing rates, PLM resulted in the greatest decrease in NO_3^- leaching, with 64% in the first year and 41% in the second year. The decreased NO_3^- leaching was associated with increased plantain content, enhanced herbage N uptake, reduced UN excretion of dairy cows and a lower N load in urine patches.

In conclusion, in a typical practice, as in the present study, incorporating 30–50% plantain with RGWC pasture decreases NO_3^- leaching from pastoral systems without adversely impacting farm productivity for at least two years from sowing. However, the reduction of plantain content in the second year suggests further measurements to determine the effectiveness of plantain-based pasture in the longer term. In the conditions and time scale of the present study, the medium plantain rate treatment (PLM) is suggested to achieve a high effectiveness of plantain incorporation in reducing NO_3^- leaching.

[1] Plantain-based pasture in this thesis refers to the mixed pasture of plantain, perennial ryegrass, and white clover.

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ABBREVIATIONS

ADF	Acid detergent fibre
BNF	Biological nitrogen fixation
CF	Crude fat
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
h	hour
ME	Metabolisable energy
MUN	Milk urea nitrogen
N	Nitrogen
NDF	Neutral detergent fibre
NH_4^+	Ammonium
NO_3^-	Nitrate
NSC	Non-structural carbohydrates
OM	Organic matter
OMD	Organic matter digestibility
PLL	Perennial ryegrass-white clover + low plantain rate
PLM	Perennial ryegrass-white clover + medium plantain rate
PLH	Perennial ryegrass-white clover + high plantain rate
RC	Relative change
RGWC	Perennial ryegrass-white clover
SEM	Standard deviation of the means
UN	Urine nitrogen

CHAPTER 1

GENERAL INTRODUCTION

This chapter provides the general background of the research topic, followed by objectives, hypotheses and approaches of the research, and the structure of the thesis.

1.1. General background

Temperate dairy systems worldwide mainly rely on perennial ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) (RGWC) pasture because this sward persists well under grazing conditions and provides high economic performance for dairy farms (Kemp, 1999). In New Zealand, the dairy sector is one of the most important industries of the country's economy. During the 2020/2021 lactation season, New Zealand had 4.9 million cows and produced 21.7 billion litres of milk, which contributed approximately NZD 10 billion in revenue to the nation (DairyNZ and LIC, 2021). However, unirrigated RGWC pastures often produce low quality and quantity of feed in summer, leading to a feed shortage during this period. In addition, the nitrogen (N) content of RGWC pasture often exceeds the N requirement of dairy cows, thus increasing the risk of N losses to the environment (Di & Cameron, 2002). The New Zealand's dairy farms often experience an annual loss of 30–90 kg NO_3^- leaching per ha (Welten et al., 2021). To reduce the environmental impact of agricultural production, the government has developed strict regulations requiring dairy farmers to mitigate N losses from their farms (Minister for the Environment, 2020).

In recent years, plantain (*Plantago lanceolata*) has become increasingly utilised as a component in mixed swards with ryegrass and clover or as a monoculture pasture in dairy farms (Dodd et al., 2019; Wilson et al., 2020). As a forage herb that is highly tolerant of drought, plantain can offer a high quality and quantity of herbage across the lactation season, improving milk production of dairy cows, especially in the summer and autumn (Cranston et al., 2015; Stewart, 1996). Incorporating plantain with RGWC pasture has recently been reported to affect N cycling in the pasture-animal-soil system, reducing N losses from dairy farms (Al-Marashdeh et al., 2021). For instance, compared to RGWC pastures, feeding plantain may decrease urinary N (UN) excretion (Dodd et al., 2018;

Minnée et al., 2020) and increase the urine volume of dairy cows (Mangwe et al., 2019; Minnée et al., 2020). This results in a decrease in the N concentration of the urine from cows grazing plantain pastures. As a result, cows may spread UN over larger areas with lower N load to urine patches, providing opportunities for pastures to uptake more UN, reducing nitrate (NO_3^-) leaching and nitrous oxide (N_2O) emission from pastoral systems (Luo et al., 2018; Woods et al., 2018). Research suggests these effects could be related to high water and mineral content, non-structural carbohydrates, undegraded N, and bioactive compounds in plantain compared to the traditional RGWC pasture (Bryant et al., 2019; Minneé et al., 2019). These factors may, together or apart, influence the UN excretion and dilution of dairy cows and inhibit the nitrification processes in soils (Bryant et al., 2019; Minneé et al., 2019).

Despite the potential benefits of plantain incorporation, various knowledge gaps require investigation before this technology can be extensively adopted on farms. Firstly, the long-term effectiveness of plantain on pasture productivity, milk production and NO_3^- leaching must be evaluated at the farm scale to demonstrate that the technology works as previous studies have only measured plantain effects over short periods of time or in indoor conditions and largely in autumn (Box et al., 2017; Carlton et al., 2019; Minnée et al., 2020). Secondly, the effect of incorporating increasing rates of plantain into RGWC pasture on farm productivity and NO_3^- leaching from pastoral systems needs to be measured to identify suitable proportions of plantain that provide the best environmental benefits. Therefore, it is essential to have a direct and robust measurement of the effect of plantain-based pasture on farm productivity and environmental benefits, as well as to propose suitable sowing rates at the paddock scale.

1.2. Research objectives

The present study was a part of the ***"Plantain Potency and Practice Programme"*** that aims to improve the use of plantain to save NZD 1 billion from 2031–2040 and reduce 15,000 tonnes of NO_3^- leaching from New Zealand's pastoral systems (MPI, 2021). The objective of the present research was to quantify the effects of plantain when it is incorporated with increasing rates into RGWC in grazing swards at the paddock level on:

- the yield and nutritive value of pasture, and the persistence of plantain,
- milk production, N excretion, and urine production of dairy cows; and
- nitrate leaching from pastoral dairy systems.

1.3. Hypotheses and approaches of the research

The hypothesis tested in the research was that incorporating plantain into RGWC pasture can achieve 30–70% plantain in the total dry matter yield, resulting in a 30-70% reduction in nitrate leaching from pastoral dairy systems compared to the nitrate leaching from the system with RGWC pastures. The decrease in NO_3^- leaching is associated with decreasing UN excretion and N load in urine patches of dairy cows and increasing herbage N uptake and is influenced by the proportion of plantain in the sward. In addition, incorporating plantain with RGWC pasture maintains or increases pasture and milk production, especially during the late lactation stage.

To address the research objectives, a field trial was conducted on an approximately 7-ha of rain-fed paddock at Massey University's No 4 Dairy Farm. Pasture treatments were perennial ryegrass (cv. ONE[50])-white clover (cv. Tribute) (RGWC), RGWC + low plantain (cv. Agritonic) rate (PLL), RGWC + medium plantain rate (PLM) and RGWC + high plantain rate (PLM), established with five replicate plots and grazed by dairy cows over 22 grazing events from September 2019 to December 2021. The yield, botanical composition and nutritive value of the pasture, milk yield and composition, urine and faecal N excretion, urine volume and urination frequency of dairy cows, and NO_3^- leaching from the pastoral system were measured during the experimental period.

1.4. Thesis structure

The thesis has seven chapters, prepared according to the format of the thesis with publications. The first two chapters include a general introduction (Chapter 1) and a literature review (Chapter 2). Chapters 3–6 present the results from the field trial, which have been published, submitted or are under preparation for submission as journal articles. Chapter 3 studies the yield and nutritive value of plantain-based pastures and the persistence of the plantain response to different sowing rates and seasons. Chapter 4 evaluates urine volume and urination frequency of dairy cows grazing plantain-based pastures. Chapter 5 examines the effect of the experimental pastures on milk production and N excretion of dairy cows. Chapter 6 determines the impact of plantain incorporation on NO_3^- leaching from the pastoral system. Finally, Chapter 7 presents an overall discussion to link the chapters, provides practical implications and recommends future studies.

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CHAPTER 2

LITERATURE REVIEW

Chapter 2 reviews the literature, providing the comprehensive analaysis of the current knowledge as well as identifying the gaps of the knowledge regarding the topics addressed in the thesis, including:

(i) the N cycle in pastoral systems,

(ii) plantain forage,

(iii) plantain versus milk production and N excretion of dairy cows, and

(iv) plantain versus NO_3^- leaching from pastoral dairy systems

A meta-analysis from this chapter has been published as:

Nguyen, T.T.; Navarrete, S.; Horne, D.J.; Donaghy, D.J.; Kemp, P.D. Forage plantain (*Plantago lanceolata* L.): Meta-analysis quantifying the decrease in N excretion, the increase in milk production, and the changes in milk composition of dairy cows grazing pastures containing plantain. *Anim. Feed Sci. Technol.* **2022**, *285*, 115244. https://doi.org/10.1016/j.anifeedsci.2022.115244.

2.1. Nitrogen cycle in pastoral dairy systems

Nitrogen (N) is a crucial nutrient element for plants and animals and is transferred between soil, pasture, and animals through an N cycle (Cameron, 1992). In pastoral dairy systems, the N input mainly comes from rainwater, fertilizers, biological N fixation, organic matter (OM) mineralisation, cow urine and faeces (Figure 2.1). Nitrogen presents in different forms in soils, such as organic matter, soil organisms or mineral-N. In soil, ammonium (NH4+) ions can react with oxygen in the nitrification process to produce nitrate (NO_3^-). Then, NO_3^- can be converted to gaseous-N (mainly nitrous oxide (N_2O)) through the denitrification process. Both NH_4^+ and NO_3^- can be taken up by plants; however, a significant amount of these N sources can be lost through drainage water or air (Cameron, 1992).

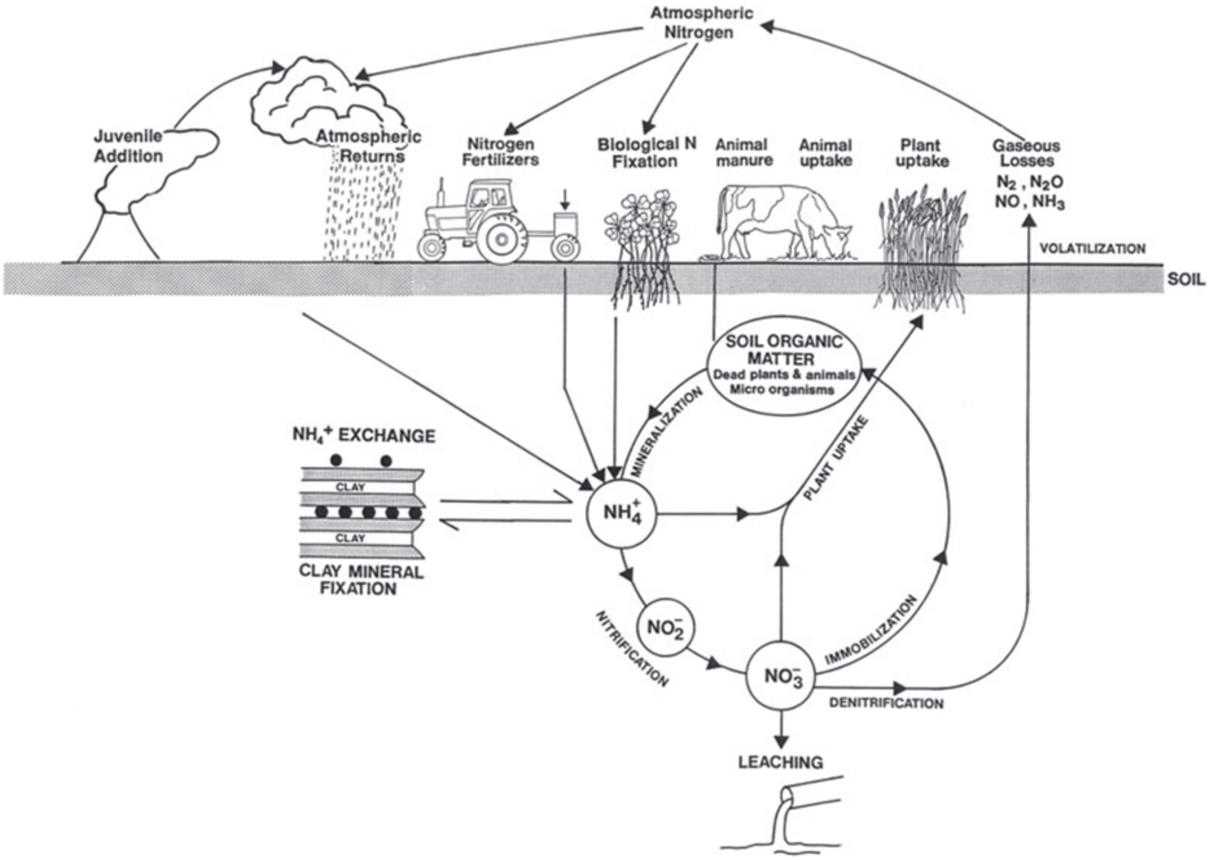

Figure 2.1. Nitrogen cycle in pastoral dairy systems

Source: Cameron (1992)

In the New Zealand pastoral dairy system, the primary source of N losses comes from urine patches deposited into paddocks by grazing cows (Selbie et al., 2015). This is because the typical dairy pastoral system with perennial ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) (RGWC) offers more N content than what is required by cattle (Cameron et al., 2013). As a result, the excess N is mainly excreted in cow urine and returned to pastoral soil at a very high concentration in urine patches. These patches may cover 20–30% of the grazed area (Moir et al., 2011), with an average N load of approximately 613 kg N per ha (Selbie et al., 2015). The pasture cannot use this excessive rate of N in these patches, and the unutilized N can be lost by NO_3^- leaching and gaseous N emission (Cameron et al., 2013).

The management of N losses targets mitigating the loss of N from different parts of the N cycle into the environment (Vogeler et al., 2013). A wide range of strategies has been applied, aiming to increase N-use efficiency, such as increasing dietary N to milk, reducing N excretion in the urine of cows, increasing the N uptake by grasses, and inhibiting the denitrification of N in soils (Cameron et al., 2013; Di & Cameron, 2002). In recent years, plantain (*Plantago lanceolata*) pasture has been considered a low-cost strategy to reduce N losses from pastoral dairy systems (Navarrete S., 2018). The effect of plantain-based pasture on different components of the N-cycle, however, is not fully understood and requires further investigation before this technology can be adopted widely by dairy farmers.

2.2. Forage plantain

2.2.1. Agronomic characteristics

Plantain (*Plantago lanceolata* L.) is a forage herb that tolerates summer heat and drought (Judson & Moorhead, 2014; Stewart, 1996). It has been increasingly grown in dairy farms throughout New Zealand and the temperate world (Dodd et al., 2019; Stewart, 1996). Mixed pastures including plantain can provide valuable pasture growth for dairy farms with limited feed in late summer and autumn (Nobilly et al., 2013; Pembleton et al., 2015), potentially increasing farm productivity and profitability (Al-Marashdeh et al., 2021; Doole et al., 2021).

Plantain has a coarse root system consisting of a truncated tap root and fibrous roots that grow deeper into the soil than the roots of ryegrass, making plantain better in drought tolerance than perennial ryegrass (van Tienderen, 1992). It can be planted in different soil types, including soils suited to ryegrass and white clover, but is most suited to free-draining soils (Stewart, 1996). Moreover, plantain adapts well to poor soil fertility, especially soils with low P and K (Hoveland et al., 1976). Nitrogen fertiliser can promote leaf, root and biomass production, increasing the competitive ability of grasses more that that of plantain in mixed pastures (Kasper, 1976). In addition, plantain tolerates well many pests and diseases. Plantain moths (*Scopula rubraria*), weevils, gallmidges, flea beetles, slugs and snails are the most common pests for plantain that may require controlling (de Nooij & Mook, 1992).

2.2.2. Establishment

Plantain can be sown as a special-purpose crop or a forage component in clover, grass-based, or diverse pastures (Dodd et al., 2019; Stewart, 1996). The establishment of plantain is described well in Powell et al. (2007) and Edwards and Pinxteruis (2022). In short, the recommended sowing time is in the autumn or spring when the soil is warm and moist for young plantain to establish. When established in new pastures, the sowing rate can be varied between 1–10 kg plantain seed/ha, depending on whether it is sown in a mixed pasture or as a monoculture. In addition, plantain seed can be directly drilled or broadcast into existing swards after hard grazing or mowing (Bryant, Dodd, et al., 2019).

2.2.3. Grazing management

Grazing management is vital to promote the establishment and improve the survival and persistence of plantain in grazing swards. The first grazing should be conducted when plantain plants have developed six full leaves, or typically 7–8 weeks after sowing, to promote the development of the root systems (Powell et al., 2007). After that, plantain or plantain-based pasture should be managed by rotational grazing with 2– 6-week intervals with a suggested pre-grazing height of 15–25 cm and post-grazing height of 7 cm to optimise pasture persistence and feed quality (Lee et al., 2015; Navarrete et al., 2013). In mixed swards, animals normally prefer to select plantain and graze it harder than ryegrass due to its high palatability; therefore, offering sufficient feed is essential to prevent damage to plantain (Somasiri et al., 2020). During late spring and summer, plantain produces a significant amount of reproductive stem and seed head. Frequent grazing or mowing may be required to maintain pasture quality (Navarrete, 2015).

2.2.3. Herbage production

The yield of plantain is vastly varied in existing literature yielding up to 20 tons/ha and is usually comparable to the RGWC pasture (Stewart, 1996). Pure plantain can produce more dry matter (DM) yield than RGWC in good conditions, especially in the first two years from sowing (Minnee et al., 2013; Moorhead & Piggot, 2009). By seasonal distribution, plantain often has a similar DM production in autumn and spring, and can produce a greater DM yield in late summer and autumn compared to ryegrass-based pastures (Moorhead & Piggot, 2009). In mixed swards, plantain-based pasture has been reported to have similar or higher yields than ryegrass-based pasture, depending on the combined components. For instance, integrating plantain with RGWC pasture can maintain annual and seasonal DM yields (Al-Marashdeh et al., 2021). However, diverse swards containing more pasture species, including plantain, have shown higher DM yield than RGWC pasture (Nobilly et al., 2013; Pembleton et al., 2015; Woodward et al., 2013).

2.2.4. Persistence

In grazing conditions, plantain can establish and develop well in the first two years after sowing; however, its population often decreases and is replaced by perennial ryegrass, white clover and weeds in older swards (Edwards & Pinxteruis, 2022). The persistence of plantain in grazing pastures depends on various factors in which plantain can persist well under low fertility levels, drying conditions, and in pastures combining less aggressive species (Stewart, 1996). In mixed swards with RGWC, plantain can contribute 30–60% of DM yield in years one and two, yet rarely accounts for more than 20% from year 3 (Dodd et al., 2019; Stewart, 1996). This is because plantain may have a relatively short lifespan and a low-competitive capacity compared to perennial ryegrass and white clover (Tiley & France, 1990). According to Bryant, Dodd, et al. (2019), plantain-based pastures reached the highest plantain content at 432–470 days after sowing. Resowing plantain seed into existing pastures might be an option to increase the percentage of plantain. However, this method is not likely to achieve more than 30% plantain in mixed pastures (Bryant, Dodd, et al., 2019).

2.2.5. Nutritive value

Plantain contains high concentrations of minerals and bioactive compounds: aucubin, acteoside and catalpol (Navarrete et al., 2016; Sanderson et al., 2003b). Compared to perennial ryegrass, plantain has lower DM content, neutral and acid detergent fibre (ADF and NDF), and soluble and degradable N; and contains more non-structural carbohydrates (NSC) and lignin (Box et al., 2017a; Minneé et al., 2019). In a recent meta-analysis, Minneé et al. (2019) reported that plantain had a 30% lower DM content, a 33% lower NDF, a 30% higher NSC, and a 26% greater mineral content compared to perennial ryegrass. In addition, plantain cv. Tonic has been reported to contain 0.44–6.87 g/kg DM of aucubin and 0.5–41.7 g/kg DM of acteoside (Navarrete et al., 2016). The nutritive value of plantain can be affected by various factors. For example, plantain can produce a higher quantity and quality of forage than ryegrass during summer and autumn due to its drought and heat-tolerant capacity (Cranston et al., 2016; Minneé et al., 2019; Nobilly et al., 2013). Fertiliser rate, seasonal and harvesting time have been reported to influence the content of minerals, bioactive compounds and nutritive value of plantain (Box et al., 2017a; Tamura, 2001). The inherent difference between plantain and ryegrass might change the nutritive value traits in mixed pastures compared with RGWC.

2.3. Plantain versus milk production and N excretion of dairy cows

The effect of plantain on milk production and N excretion of dairy cows has been comprehensively reviewed in a meta-analysis which is provided in Appendix 1 (Nguyen et al., 2022). The meta-analysis used the data from 12 studies to compare milk yield and milk composition, UN concentration in urine, urine volume and UN excretion between cows offering the pastures with and without plantain. The key outcomes of the meta-analysis are shown in Table 2.1. This section summarises the main findings and discussions from the meta-analysis.

Table 2.1. Milk production and urinary nitrogen excretion of dairy cows offered pastures with and without plantain.

Variable	Lactation stage	N	WMD	95% CI	I^2 (%)	P
Milk production						
Milk yield (kg/cow/day)	Overall	26	1.02	0.55–1.46	79	< 0.001
	Early lactation	7	0.36	-0.33–1.05	50	0.407
	Late lactation	18	1.38	0.83–1.94	55	< 0.001
Milk solids (kg/cow/day)	Overall	20	0.07	0.02–0.12	68	0.012
	Early lactation	5	0.02	-0.09–0.05	0	0.545
	Late lactation	14	0.11	0.05–0.17	56	0.002
Milk protein (g/100 g milk)	Overall	26	0.02	-0.02–0.06	71	0.274
	Early lactation	7	-0.01	-0.06–0.04	57	0.575
	Late lactation	18	0.03	-0.02–0.08	58	0.162
Milk protein (g/cow/day)	Overall	26	23.4	11.3–35.5	68	< 0.001
	Early lactation	7	12.2	-22.7–47.0	70	0.410
	Late lactation	18	31.1	14.7–47.6	60	0.003
Milk fat (g/100 g milk)	Overall	26	-0.24	-0.31 to -0.17	57	< 0.001
	Early lactation	7	-0.13	-0.17 to -0.09	0	< 0.001
	Late lactation	18	-0.29	-0.38 to -0.19	55	< 0.001
Milk fat (g/cow/day)	Overall	26	6.3	-14.8–27.4	79	0.554
	Early lactation	7	-21.9	-53.9–10.1	70	0.146
	Late lactation	18	15.4	-3.5–34.2	35	0.101
UN concentration, urine volume and UN excretion						
UN concentration (ROM)	Overall	19	0.70	0.62–0.80	91	< 0.001
	Early lactation	4	0.72	0.52–0.98	40	0.044
	Late lactation	15	0.70	0.59–0.82	93	< 0.001
Urine volume (ROM)	Overall	15	1.17	1.07–1.29	85	0.003
	Early lactation	5	1.06	0.94–1.20	0	0.244
	Late lactation	10	1.19	1.05–1.36	77	0.010

UN excretion (ROM)	Overall	16	0.78	0.72–0.85	95	< 0.001
	Early lactation	4	0.73	0.58–0.92	88	0.023
	Late lactation	13	0.80	0.72–0.89	96	< 0.001

Note: UN = urinary nitrogen, ROM = ratio of means, N = the number of comparisons, WMD = weighted mean difference, CI = confidence interval, I^2 = within studies heterogeneity.

2.3.1. Milk production

Over the whole lactation year, compared to grazing RGWC pasture, including an average of 43% plantain in the diet resulted in a 1.02 kg/cow/day increase in milk yield, a 0.07 kg/cow/day increase in milk solids, and a 23.4 g/cow/day more increase in milk protein (Table 2.1, $P < 0.05$). In contrast, feeding pastures containing plantain led to a decrease in milk fat concentration by 0.24 g/100 g milk ($P < 0.05$). Milk fat yield and protein concentration were similar between cows fed pastures with and without plantain. The effect of plantain inclusion on increasing milk production has been suggested to be due to improved DM intake (DMI) and herbage quality (Minnée et al., 2020; Wilson et al., 2020). In the meta-analysis, the DMI of cows with the pasture containing plantain was higher than with the control pasture by 1.03 kg/cow/day. In addition, the experimental diet had lower ADF and NDF than the control pasture, allowing cows to consume and digest more feed in the same period. The higher DMI can result in a greater metabolisable energy intake to produce more milk. The only potentially negative impact of feeding plantain reported has been a depression in milk fat concentration (Dodd et al., 2018; Minnée et al., 2020). The critical driver for reducing fat concentration in milk may relate to differences in NDF content between plantain and ryegrass. The low NDF content in plantain could lead to an insufficient supply of acetate for de novo fat production, depressing milk fat concentration (Palmquist et al., 1993).

The effect of plantain on milk production and milk composition differed between lactation periods. When plantain was included in the diet, most milk parameters were increased in late lactation and were maintained in early lactation. Specifically, in the late lactation, cows grazing the pasture containing plantain had a higher milk yield, solids yield, and protein yield than those grazing the control pasture ($P < 0.05$). In contrast, there were no differences in milk yield, solids, protein concentration, and protein yield in early lactation ($P > 0.05$). In addition, the milk fat concentration of cows grazing the treatment pasture was lower than those grazing the control pasture in both early and late lactation periods. The difference in DMI and forage quality was more remarkable in late lactation than in early lactation, causing a more significant effect on milk production. In late lactation, the decline in the nutritive value of RGWC is often a limit to milk production (Kemp, 1999). Plantain can maintain its nutritive value during summer and autumn due to its drought and heat tolerance. In contrast, the sound growing conditions in spring and early summer led to less difference in nutritive value between plantain and RGWC that was not large enough for a measurable effect of plantain on milk production.

2.3.2. UN concentration, urine volume and UN excretion

Overall, including plantain in the diet reduced UN concentration by 30%, decreased UN excretion by 22%, and increased urine volume by 17%, compared to grazing RGWC pastures (Table 2.1, $P < 0.05$). The effect of plantain inclusion on reducing UN concentration and UN excretion was found in both early and late lactation stages. However, the impact of plantain pastures on daily urine volume was reported to be significantly increased only in late lactation. A key driver for the lower UN concentration in cows eating plantain is a higher proportion of rumen-undegradable N (Bryant, Snow, et al., 2019; Minnée et al., 2020). A greater undegradable N content allows more N to pass through the rumen to be digested in the small intestine, where more N is partitioned to faeces (Wilson et al., 2020), and less N is excreted into urine (Bryant et al., 2018). Moreover, the high contents of water and minerals in plantain potentially increase water intake and urine volume of dairy cows (Mangwe et al., 2019; Minneé et al., 2019). Furthermore, the difference in DMI between plantain and ryegrass during summer and autumn is higher than in spring, resulting in a more significant effect of plantain on urine volume in late lactation (Box et al., 2017b; Dodd et al., 2018). In addition, the reduction in UN excretion may also to be attributed to the presence of aucubin in plantain. Aucubin is known to be associated with a reduction in ammonia production in the rumen fluid (Navarrete et al., 2016).

Meta-regression analysis for the effect of the content of plantain in the treatment pasture indicated that increased plantain content was statistically associated with a reduction in UN concentration, a decrease in UN excretion, and an increase in urine volume. Most individual studies agree that diets containing 20% plantain or less do not significantly affect UN concentration (Minneé et al., 2017), urine volume (Bryant et al., 2018), and total UN excretion (Minnée et al., 2020). The effective proportion of plantain has been reported in previous studies that was 30% for reducing UN concentration, 45% for increasing urine volume (Minnée et al., 2020), and 50% for decreasing UN excretion (Box et al., 2017b). The meta-regression using the data from individual studies estimated curves for the relative changes (RC) of UN concentration, urine volume and UN excretion as shown below:

- RC of UN concentration (%): $y = -0.0645 \times$ plantain content (g/kg DM) $+ 0.61$
- RC of urine volume (%): $y = 0.0659 \times$ plantain content (g/kg DM) $- 12.73$
- RC of UN excretion (%): $y = -0.0297 \times$ plantain content (g/kg DM) $- 5.86$

2.4. Plantain versus NO_3^- leaching

Plantain has been increasingly integrated into grazing pastures in recent years to reduce NO_3^- leaching from dairy systems. Lysimeter studies have revealed that incorporating plantain with traditional grasses can decrease NO_3^- leaching from urine patches by up to 89% (Carlton et al., 2019; Woods et al., 2018). At the farm scale, NO_3^- leaching from pure plantain has shown to be 48% lower in the first year from the establishment period and similar in the second year compared to RGWC pasture (Rodriguez, 2020). Moreover, the author found no advantages of plantain-clover mix in reducing NO_3^- leaching from pastoral dairy systems. In addition, a modelling study indicated that incorporating plantain with RGWC can reduce NO_3^- leaching from dairy systems by 56% (Al-Marashdeh et al., 2021). The lower NO_3^- leaching has been associated with increasing the content of plantain and decreasing clover content in the swards (Rodriguez, 2020). Unfortunately, no studies have directly quantified the effect of plantain-based pasture on NO_3^- leaching at the farm level.

Plantain can influence different N components of the animal-pasture-soil system, reducing NO_3^- leaching from pastoral dairy farms (Bryant, Snow, et al., 2019). For instance, plantain has been found to enhance the rumen synchrony of energy and protein and decrease the protein degradability. This, in turn, increases the partitioning of dietary N to milk and dung, while reducing N excretion in urine (Marshall et al., 2021; Minnée et al., 2020). Moreover, the high water content in plantain has been shown to increase urine volume and urination frequency, spreading UN over a greater area with a lower UN load (Mangwe et al., 2019; Minnée et al., 2020). Consequently, pastures may uptake more N from urine patches, decreasing NO_3^- leaching in drainage water (Woods et al., 2018). In addition, bioactive compounds such as aucubin and acteoside present in plantain have been shown to reduce ammonia production in cow rumen (Navarrete et al., 2016) and inhibit NO_3^- production in pastoral soils (Gardiner et al., 2017; Judson et al., 2019; Rodriguez et al., 2021).

2.5. Knowledge gaps

There is a demand to fully understand the effect of plantain incorporation on productivity and nutritive value of pastures, farm productivity, and environmental benefits as this sward becomes more commonly used on farms. Existing literature has intensively studied the yield and quality of pure plantain (Box et al., 2018; Lee et al., 2015; Sanderson et al., 2003a). However, there have been limited data on the effect of plantain-based pasture on DM yield and herbage nutritive value. In addition, previous studies have determined the impact of plantain-based pasture on animal performance in short-term studies and NO_3^- leaching at lysimeter scale (Woods et al., 2018) or by modelling (Al-Marashdeh et al., 2021); however, the long-term effect at farm level is not fully understood. Therefore, a credible and comprehensive measurement of the DM production and nutritive value of the pasture incorporating increasing rates of plantain and its effects on milk production, UN excretion and urination behaviour of dairy cows, and NO_3^- leaching from pastoral systems will provide robust evidence to encourage adoption of plantain on farms.

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CHAPTER 3

IMPACT OF PLANTAIN INCORPORATION ON DRY MATTER YIELD, PLANTAIN PERSISTENCE AND NUTRITIVE VALUE OF PASTURE

Incorporating plantain into perennial ryegrass-white clover pasture is hypothesized to improve pasture production and animal performance and decrease NO_3^- leaching from pastoral systems. This chapter quantifies the effect of incorporating increasing rates of plantain with RGWC on the first aspect, including the yield and the nutritive value of the pastures and the persistence of plantain.

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Abstract

Incorporating plantain into the traditional perennial ryegrass-white clover (RGWC) pasture can improve the quality and quantity of grazing pastures; however, the sowing rate could affect the persistence of plantain, pasture yield, and nutritive value in different seasons. The objective of the present study was to evaluate the effect of increasing sowing rates of plantain when established with RGWC on dry matter yield (DM), botanical composition, nutritive value, and bioactive compounds of the pasture over the first two years after sowing. Moreover, the study determined the relationship between plantain content and nutritive characteristics in different seasons. The pasture treatments were RGWC, RGWC + low plantain rate (PLL), RGWC + medium plantain rate (PLM), and RGWC + high plantain rate (PLH). The results showed that annual DM yield was similar between treatments. The average plantain proportion (including leaves and reproductive stem) in the pastures was 32, 44, and 48% in PLL, PLM, and PLH, respectively. The plantain composition increased in the first 15 months, then declined rapidly to 20% in PLL and 30% in PLM and PLH at day 705 after sowing. Compared with the RGWC pasture, the plantain-based pastures (PLL, PLM, PLH) had a higher content of organic matter digestibility, ash, starch, non-structural carbohydrates, phosphorus, sulfate, calcium, magnesium, sodium, chloride, zinc, boron, cobalt, aucubin, acteoside, and catalpol. In addition, plantain incorporation into RGWC reduced DM content, acid detergent fibre, neutral detergent fibre, crude fat, iron, and manganese in the pasture. These differences were linearly associated with plantain content and were higher in summer and autumn than in spring. In conclusion, incorporating plantain with RGWC pasture can improve herbage nutritive quality, thus potentially increasing farm productivity and environmental benefits. However, further work is required to investigate management interventions to sustain plantain content beyond two years from sowing.

3.1. Introduction

Plantain (*Plantago lanceolata*) is a forage herb highly tolerant of summer heat and drought (Judson & Moorhead, 2014; Stewart, 1996). Since the 2000s, plantain has increasingly grown in mixed swards in temperate dairy grazing farms (Moorhead & Piggot, 2009). Incorporating plantain into grazing pastures can improve the quality and quantity of feed, especially in the late summer and autumn (Nobilly et al., 2013; Pembleton et al., 2015), thereby potentially increasing farm productivity and profitability (Al-Marashdeh et al., 2021; Doole et al., 2021). Moreover, including plantain in grazing pastures has been considered an effective forage strategy to reduce nitrate (NO_3^-) leaching from pastoral dairy systems to meet the strict regulations of local governments (Al-Marashdeh et al., 2021; Minister for the Environment, 2020). While perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) (RGWC) are the mainstays of temperate dairy pastures (P.D. Kemp, 2000), incorporation of plantain appeals to pastoral dairy farmers because of its advantages in reducing nitrogen (N) losses from the system. Various studies have measured the yield and nutritive value of pure plantain (Box et al., 2018; Lee et al., 2015) or diverse swards containing plantain (Nobilly et al., 2013; Pembleton et al., 2015). However, there has been limited understanding of the dry matter (DM) yield and herbage nutritive value of pastures incorporating plantain with RGWC.

Plantain has greater non-structural carbohydrates (NSC) concentration and lower structural carbohydrates than the RGWC pasture (Minneé et al., 2019). Therefore, feeding pastures containing plantain can improve the DM intake of dairy cows and provide more readily available energy for microbes to produce microbial N for milk production (Minneé et al., 2017). Furthermore, high concentrations of minerals (Harrington et al., 2006), bioactive compounds aucubin and acteoside (Navarrete et al., 2016), and water contents (Minneé et al., 2019) in plantain have been implicated in a reduction of urine N concentration and an increase in urine volume of dairy cows. Therefore, the inclusion of plantain can lower UN excretion by dairy cows (Minnée et al., 2020), contributing to a lower risk of NO_3^- leaching (Carlton et al., 2019) and nitrous oxide emission (Simon et al., 2019). Research has suggested that ryegrass-based pastures may need at least 30% dietary plantain to have measurable effects on animal performance and N uses efficacy (Minnée et al., 2020; Nkomboni et al., 2021). Furthermore, an increased plantain content in the diet can result in a greater reduction in UN excretion (Carlton et al., 2019; Minnée et al., 2020). However, plantain composition in diverse pastures has been reported to decline rapidly beyond two years from sowing (Dodd et al., 2019). Therefore, it is necessary to determine suitable sowing rates to achieve a high percentage of plantain for an extended period in the ryegrass-based pasture, while maintaining or increasing the herbage DM yield.

Various biological and environmental factors can influence the DM yield and nutritive value of plantain-based pasture. The hypothesis is that an increased plantain content is associated with greater DM yield, improved herbage nutritive value, and the content of minerals and bioactive compounds. Moreover, the relationships between plantain content and nutritive value traits vary in different seasons. Specifically, as a drought and heat-tolerant herb, plantain may produce a greater quantity and quality of forage than RGWC during summer and autumn (Cranston et al., 2016; Minneé et al., 2019; Nobilly et al., 2013). On the other hand, the contents of minerals and bioactive compounds in plantain-based pastures are greater than in RGWC pastures, especially in drier seasons (Metson & Saunders, 1978; Navarrete et al., 2016; Tamura, 2001).

The objective of the current study was to compare the herbage DM yield, botanical composition, nutritive value, and the contents of minerals and bioactive compounds of the pastures sown with increased plantain seed rates and grazed with dairy cows. Furthermore, the study aimed to illustrate the change in plantain content of the pastures throughout two years from sowing, and to estimate the relationship between plantain content and the herbage nutritive value traits of the pasture in different seasons.

3.2. Materials and methods

3.2.1. Study site, pasture treatment, and establishment

The study was conducted at Massey University's No 4 Dairy Farm (40°23′26.9″S, 175°36′43.5″E) in Palmerston North, New Zealand, between April 2019 and March 2021. The soil at the experimental site is Tokomaru silt loam soil. The soil analysis to 7.5 mm reported content of minerals (parts per million) of 30 Olsen-phosphorus, 3.7 sulfate-sulfur, 5.9 potassium, 9.0 calcium, 31 magnesium, 4.8 sodium, and a pH of 5.8. The experimental site has a temperate climate, with an average annual precipitation of 962 ± 129 mm and a yearly temperature of 14.1 ± 0.2 °C during the study period.

Pasture treatments were four mixed swards, including RGWC, RGWC + low plantain rate (PLL), RGWC + medium plantain rate (PLM), and RGWC + high plantain (PLH). Agritonic is a recent plantain cultivar developed in a breeding program for use within grazing pasture mixes to reduce NO_3^- leaching from pastoral systems (Judson et al., 2018). The experimental pastures were sown by direct drilling on 5 April 2019 with the rates presented in Table 3.1. The existing swards were eliminated before sowing with Polaris 540 (54% glyphosate) at 4 L/ha, Harmony (50% Thifensulfuron methyl) at 30 g/ha, and Pulse penetrant (Organo-silicone penetrant) at 100 mL/ha. The experiment was a completely randomised design with five replicate plots. Twenty experimental plots of 800 m^2 (40 m × 20 m) and four adaptation areas (1-ha area per pasture treatment) were split from an approximately 7-ha paddock. No fertiliser was applied at the sowing time. In March 2020, plantain seeds were directly drilled at the rates of 3, 6, and 9 kg/ha in PLL, PLM and PLH, respectively, with the aim to improve the plantain proportion in the pastures. The pasture was mowed after the grazing in October 2019, January 2020, October 2020, and December 2020 to control the pasture seed head and to equilibrate the post-grazing residual height between plots. The mowing was conducted using a Tiger forage harvester and defoliating to 7 cm height. Urea fertiliser (46% N) was applied at 50 kg N/ha in October 2019, December 2019, and February 2020, and 30 kg N/ha in September 2020 and November 2020.

Table 3.1. Cultivars and sowing rates of pasture treatments.

Species/Cultivar	**Sowing Rate (kg seed/ha)**			
	RGWC	PLL	PLM	PLH
Plantain (Agritonic)	0	4	7	10
White clover (Tribute)	3	3	3	3
Perennial ryegrass (ONE^{50})	20	15	10	5

Note: RGWC = perennial ryegrass-white clover, PLL = RGWC + low plantain rate, PLM = RGWC + medium plantain rate, and PLH = RGWC + high plantain rate.

3.2.2. Grazing Management

The experimental pasture was grazed by dairy cows over two lactation years, from September 2019 to March 2021. The grazing rotation was at 3–6-week intervals, equating to 9 grazings in the 2019/2020 and 7 grazings in the 2020/2021 lactation years (Table 3.2). In addition, a short grazing event was conducted in four hours with 100 dry cows in week 12 after sowing to graze the top of the ryegrass pasture to promote plantain establishment. Pasture cover was managed at under 4000 kg DM/ha for pre-grazing mass and above 1300 kg DM/ha for post-grazing mass. In each grazing, either 3 or 4 cows were allocated for grazing from 1.5–3 days in each plot after having 6 days of grazing in the adaptation areas with the same pasture type.

Table 3.2. Season, grazing period, number of cows and days grazing in each plot and measurements for grazing events during the experimental period.

	2019/2020			2020/2021		
Season	**Grazing period**	**Cow × day/plot**	**Measurement**	**Grazing period**	**Cow × day/plot**	**Measurement**
Spring	18–20 Sep	4 × 2	DM, BC, NV	2–5 Sept	3 × 3	DM, BC, NV
	22–24 Oct	4 × 2	DM, BC	12–15 Oct	4 × 3	DM, BC, NV
	19–21 Nov	4 × 2	DM, BC	16–19 Nov	4 × 3	DM, BC, NV
Summer	10–12 Dec	4 × 2	DM, BC, NV, BA	8–10 Dec	3 × 2	DM, BC, NV, BA
	14–17 Jan	4 × 3	DM, BC	11–14 Jan	4 × 3	DM, BC
	11–12 Feb	3 × 1.5	DM, BC, NV, BA	8–10 Feb	3 × 2	DM, BC, NV, BA
Autumn	17–19 Mar	3 × 2	DM, BC, NV, BA	29–31 Mar	3 × 2	DM, BC, NV, BA
	12–15 May	3 × 3	DM			
Winter	6–10 Jul	3 × 4	DM, BC			

Note: DM = dry matter growth, BC = botanical composition, NV = nutritive value and the content of minerals, and BA = bioactive compounds.

3.2.3. Herbage measurement

The period of measurements for herbage DM yield, botanical composition, nutritive value, along with the content of minerals and bioactive compounds of the pastures, are presented in Table 3.2. Herbage yield and botanical composition were measured for every grazing event over the experimental period. Nutritive value, along with the content of minerals, was measured for four grazings in the first lactation year (September 2019, December 2019, February 2020, and March 2020) and seven grazings in the second lactation year (September 2020, October 2020, November 2020, December 2020, February 2020, March 2020, May 2020). In addition, the concentration of bioactive compounds was measured for all grazing events with the measurement of nutritive value, except grazing in September 2019, September 2020, October 2020, and November 2020.

Pre-grazing and post-grazing herbage DM mass was measured for individual plots in every grazing. In each grazing, three herbage samples (0.1 m^2 quadrat) were randomly cut to ground level from each plot a day before and after the grazing, using an electric shearing handpiece. Herbage samples were cleaned by manually removing soil and faecal debris and dried in an oven at 75 °C until a constant weight was achieved. Herbage DM production between two consecutive grazing events was estimated by deducting the average post-grazing DM mass of the previous grazings from the average pre-grazing DM mass of the current grazings.

Botanical composition and herbage nutritive value were measured from hand-plucked samples collected a day before the grazing in individual experimental plots. A hand-plucked sample (approximately 400 g fresh weight) was collected from each plot in every grazing with 15–20 random grabs to the grazing height at approximately 5-7 cm. Each sample was mixed thoroughly and divided into two sub-samples for botanical composition separation and herbage nutritive value analysis. The botanical composition samples (approx. 100 g fresh weight) were manually separated into ryegrass, white clover, plantain leaves, plantain reproductive stem and seed head, weeds, and dead materials. These components were dried in an oven at 75 °C until a constant weight was achieved to calculate the botanical composition on a dry weight basis. The herbage nutritive value sub-samples were weighed to record the fresh weight and then oven-dried at 60 °C until a constant weight was achieved to determine DM content. Then, the dried samples were ground through a 1 mm sieve for chemical analyses.

Analytical testing for nutritive value was conducted by a commercial laboratory using near-infrared (NIR) spectroscopy (Hill Lab, 2021). The samples were analysed for organic matter digestibility (OMD) (in-vivo using the Australian Fodder Industry Association method), crude protein (CP) (N multiplied by 6.25), acid detergent fibre (ADF), neutral detergent fibre (NDF), lignin, ash, metabolisable energy (ME) (calculated from OMD), soluble sugars, starch, crude fat (CF) and NSC (100 - CP - ash - CF - NDF). In addition, the samples were analysed for the content of N, phosphorus, potassium, sulphur, calcium, magnesium, sodium, chloride, iron, manganese, zinc, copper, boron, molybdenum, cobalt, selenium, ryegrass staggers index and dietary cation-anion difference. The analysis for aucubin, acteoside, and catalpol was conducted by high-performance liquid chromatography at Massey University. The detail of this method was described by Navarrete et al. (2016).

3.2.4. Statistical analysis

Annual DM yield was obtained by sum up the accumulated DM growth of grazing events occurring in the lactation years. Data on the DM yield, botanical compositions, nutritive value, and content of minerals and bioactive compounds over the experimental period were analysed using PROC mixed procedure in SAS (SAS Institute, 2020). Pasture treatment, season, and the interaction between treatment and season were fixed effects. Replicate plot and year were random effects. Means among treatments were compared using Fisher's least significant difference test. A significant difference between means was declared at $P < 0.05$.

The change in plantain content (percentage of pastures composed of plantain) of the pasture treatments from the first grazing to 705 days after sowing (between September 2019 and March 2021) was analysed according to a modified Gaussian functional model:

$y = p \times \exp(-0.5 \times ((day - d)/s)^2$ (Archontoulis & Miguez, 2015). Where p is the peak of plantain content, d is the peak day; and s is the coefficient controlling the peak width (slope). The significant difference in p, d, and s between treatments was declared when 83.4% confidence intervals did not overlap (Julious, 2004).

The relationships between the content of plantain leaves and parameters related to nutritive values, the contents of minerals and bioactive compounds were estimated for different seasons using a PROC GLM procedure in SAS (SAS Institute, 2020). The model was $y = \alpha + \beta \times P_j + S_i + e_{ij}$. This model included an intercept (α), a slope of plantain effect (β), the content of plantain leaves of a plot in a grazing event (P_j), and the season (S_i, i = spring, early summer, late summer, or autumn). The model was presented for all parameters with a significant effect of the plantain leaves (%) in the pasture ($P < 0.05$). The impact of plantain leaves (%) and season (P), the coefficient of determination (R^2), and the root mean square error (r.m.s.e) were presented for each generated model. A significant effect of plantain content and season was declared at $P < 0.05$.

3. Results

3.3.1. Herbage yield

There was no significant difference in the annual DM yield between the pasture treatments over the experimental period (Table 3.3 and Appendix 2). Under a dairy grazing system, the yearly yield across treatments was approximately 14,500 kg DM/ha. Mean pre-grazing DM mass and post-grazing DM mass of the pastures were managed at about 3,400 and 1,700 kg DM/ha, respectively. Pre-grazing DM mass of the pastures was not significantly different among treatments; however, post-grazing DM mass of the pastures in PLL, PLM and PLH was lower compared to that in RGWC ($P < 0.05$).

Table 3.3. Pasture production, botanical composition, nutritive value, minerals, and bioactive compounds in pasture treatments over the experimental period.

Variable	**RGWC**	**PLL**	**PLM**	**PLH**	**SEM**	**P_{T}**	**P_{S}**	**$P_{T\times S}$**
Pasture production								
Pre-grazing mass (kg DM/ha)	3,466	3,418	3255	3,356	424	0.263	0.189	0.420
Post graze mass (kg DM/ha)	$1,875^{a}$	$1,761^{b}$	$1,571^{c}$	$1,661^{bc}$	451	0.006	<0.001	0.056
Annual DM yield (kg DM/ha)	14,303	14,361	14,407	14,999	1981	0.495	-	-
Botanical composition								
Plantain leaves (%)	3.3^{a}	23.8^{b}	34.5^{c}	37.6^{c}	3.0	<0.001	0.599	0.090
Plantain stem (%)	1.2^{a}	7.9^{b}	9.5^{b}	10.2^{b}	2.1	<0.001	0.345	<0.001
Perennial ryegrass (%)	62.7^{a}	37.7^{b}	27.6^{c}	25.2^{c}	7.4	<0.001	0.024	0.145
White clover (%)	16.1	14.0	15.3	14.5	5.8	0.842	0.002	0.516
Weeds (%)	1.1	1.3	0.8	1.1	0.6	0.759	0.144	0.814
Dead material (%)	16.3^{a}	13.7^{b}	10.8^{c}	10.0^{c}	1.4	<0.001	<0.001	0.014
Nutritive value								
DM (%)	27.0^{a}	22.8^{b}	20.8^{c}	20.5^{c}	2.1	<0.001	<0.001	<0.001
OMD (g/kg DM)	701^{a}	713^{a}	734^{b}	726^{ab}	14	<0.001	0.027	0.007
CP (g/kg DM)	186	188	194	192	23	0.333	0.924	0.750
ADF (g/kg DM)	269^{a}	265^{a}	254^{b}	255^{b}	6.5	0.003	0.027	0.427
NDF (g/kg DM)	465^{a}	440^{b}	403^{c}	405^{c}	10	<0.001	0.002	0.987
Lignin (g/kg DM)	77	81	86	90	5.9	0.103	0.018	0.342
Ash (g/kg DM)	109^{a}	111^{ab}	118^{b}	115^{b}	9.5	<0.001	0.678	0.297
Soluble sugars (g/kg DM)	72	68	70	67	11	0.671	0.018	0.003
Starch (g/kg DM)	7.7^{a}	10^{ab}	11^{b}	13^{b}	1.7	0.009	0.673	0.709
CF (g/kg DM)	28^{a}	27^{ab}	26^{bc}	25^{c}	0.5	<0.001	<0.001	0.317
NSC (g/kg DM)	211^{a}	232^{b}	258^{c}	253^{c}	19	<0.001	0.055	0.988
ME (MJ/kg DM)	10.0	10.2	10.4	11.1	0.9	0.683	0.171	0.894
Mineral content								
Phosphorus (g/kg DM)	3.5^{a}	3.8^{b}	3.9^{c}	3.9^{c}	0.2	<0.001	<0.001	0.038
Potassium (g/kg DM)	23.5	23.7	24.2	24.6	1.8	0.699	<0.001	0.470
Sulfur (g/kg DM)	2.8^{a}	3.1^{b}	3.3^{c}	3.3^{c}	0.1	<0.001	0.492	0.094
Calcium (g/kg DM)	8.4^{a}	11.9^{b}	14.6^{c}	14.6^{c}	0.7	<0.001	0.277	0.546
Magnesium (g/kg DM)	2.6^{a}	2.7^{ab}	2.8^{b}	2.8^{b}	0.1	<0.001	0.001	0.287
Sodium (g/kg DM)	3.6^{a}	5.0^{b}	5.9^{b}	5.7^{b}	0.5	<0.001	0.154	0.072
Chloride (g/kg DM)	12.8^{a}	15.7^{b}	17.2^{b}	17.0^{b}	1.9	<0.001	0.711	0.052
Iron (mg/kg DM)	242^{a}	184^{b}	162^{b}	162^{b}	39	<0.001	0.032	0.181
Mangaese (mg/kg DM)	125^{a}	115^{ab}	109^{ab}	95^{b}	14	0.025	0.125	0.297
Zinc (mg/kg DM)	36^{a}	42^{b}	46^{c}	46^{c}	2.5	<0.001	0.071	0.553
Copper (mg/kg DM)	11^{a}	13^{b}	14^{b}	14^{b}	2.5	<0.001	0.143	0.002
Boron (mg/kg DM)	12^{a}	16^{b}	19^{c}	19^{c}	2.4	<0.001	0.468	0.676
Cobalt (mg/kg DM)	0.43^{a}	0.44^{a}	0.43^{a}	0.59^{b}	0.16	<0.001	0.266	<0.001
Selenium (mg/kg DM)	0.03	0.03	0.04	0.03	0.00	0.058	0.197	0.213
DCAD (meq/kg DM)	228	204	191	195	41	0.129	0.209	0.008
Bioactive compound								
Aucubin (g/kg DM)	0.69^{a}	3.78^{b}	5.76^{c}	6.58^{c}	2.00	<0.001	0.249	0.136
Acteoside (g/kg DM)	0.68^{a}	4.79^{b}	8.01^{c}	8.89^{c}	0.79	<0.001	0.090	0.131
Catalpol (g/kg DM)	0.38^{a}	0.69^{b}	0.80^{b}	0.80^{b}	0.31	0.002	0.158	0.087

Note: $^{a, b, c, d}$ means within a row with different superscripts differ at $P < 0.05$, ADF = acid detergent fibre, CF = crude fat, CP = crude protein, DCAD = dietary cation-anion difference, DM = dry matter, ME = metabolisable energy, NDF = neutral detergent fibre, NSC = non-structural carbohydrate, RGWC = perennial ryegrass-white clover, PLL = RGWC + low plantain rate, PLM = RGWC + medium plantain rate, PLH = RGWC + high plantain rate, OMD = organic matter digestibility, SEM = standard of the mean, T = treatment, S = season.

3.3.2. Botanical composition

The composition of plantain leaves, plantain reproductive stems and seed heads, ryegrass, and dead materials differed between treatments over the experimental period ($P < 0.05$, Table 3.3). The contents of plantain leaves in PLH (37.6%) and in PLM (34.5%) were higher than in PLL (23.8%) ($P < 0.05$). In addition, the plantain-based pastures (PLL, PLM, PLH) had between 7.9 and 10.2% plantain reproductive stem. The greater content of plantain resulted in a lower composition of perennial ryegrass and dead materials in the swards. Perennial ryegrass and dead material accounted for 25.2% and 10% in PLH, 27.6% and 10.8% in PLM, and 37.7% and 13.7% in PLL, respectively. Those figures were lower than that of RGWC (62.2% and 16.3%) ($P < 0.05$). No significant difference in the composition of white clover and weeds was observed among treatments over the experimental period.

The Gaussian models estimated that the total plantain content (including leaves and reproductive stem) increased over the first year to peak at 46.5% in PLL on day 442, 63.0% in PLM on day 455, and 64.2% in PLH on day 421 after sowing. Then, the proportion of plantain declined rapidly to approximately 20% in PLL and 30% in PLM and PLH on day 705 after establishment (Table 3.4 and Figure 3.1). The peaks of plantain content in PLM and PLH were similar, and those figures were significantly higher than in PLL. The time for PLH to achieve the highest plantain content (day 421) was statistically earlier than for PLM (day 455). No difference in the slope of the models was found in our analysis.

Table 3.4. Gaussian models to estimate plantain content in pasture treatments from day 150 to 705 after sowing.

Parameter	PLL			PLM			PLH		
	Mean	SE	83.4% CI	Mean	SE	83.4% CI	Mean	SE	83.4% CI
Peak	46.5	2.8	42.7–50.4	63.0	2.1	60.1–65.9	64.2	2.7	60.5–67.9
Day	442	11	427–458	455	7	445–465	421	9	408–433
Slope	200	16	177–222	211	10	196–225	235	16	212–258

Note: CI = confidence interval, PLL = perennial ryegrass-white clover (RGWC) + low plantain rate, PLM = RGWC + medium plantain rate, PLH = RGWC + high plantain rate, SE = standard error;

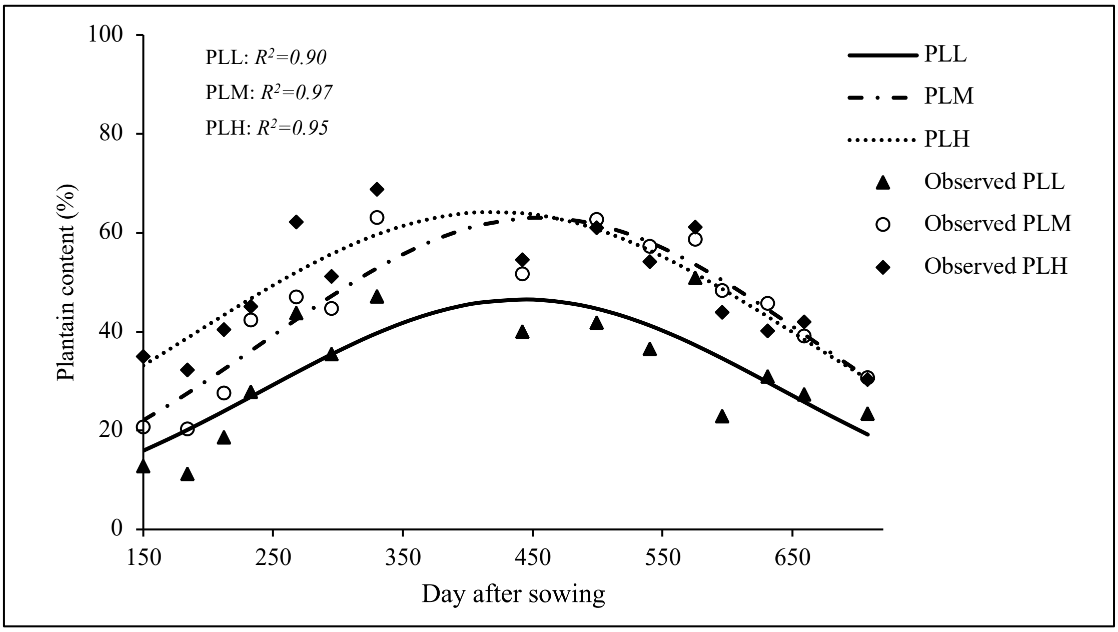

Figure 3.1. The modelled and observed percentage of plantain in pasture treatments from day 105 to 705 after sowing.

Note: PLL = perennial ryegrass-white clover (RGWC) + low plantain rate; PLM = RGWC + medium plantain rate; PLH = RGWC + high plantain rate; R^2 = coefficient of determination.

3.3.3. Herbage nutritive value and contents of minerals and bioactive compounds

Most parameters for nutritive value in Table 3.3 were significantly affected by treatment, except for CP, lignin, soluble sugars, ME, potassium, molybdenum, selenium, ryegrass staggers index, and dietary cation-anion difference. In detail, PLH and PLM were higher than RGWC in OMD, ash, starch, and NSC, but these plantain-based treatments (PLH and PLM) were lower in DM content, ADF, NDF, and CF ($P < 0.05$). In addition, PLM and PLH had a higher concentration of catalpol, aucubin, and acteoside, and most minerals (phosphorus, sulfur, calcium, magnesium, sodium, chlorine, zinc, copper, boron, cobalt) than RGWC, except for iron and manganese, which were greater in RGWC ($P < 0.05$). The same trends were observed between PLL and RGWC, with a lower difference. A significant difference between PLL and RGWC was found for DM%, NDF, NSC, phosphorus, sulfur, calcium, sodium, chlorine, zinc, copper, boron, catalpol, aucubin, and acteoside.

3.3.4. Effect of treatment in different seasons

The interaction between treatment and season significantly affected plantain reproductive stem, dead materials, DM content, OMD, phosphorus, copper, and cobalt ($P < 0.05$, Table 3.3 and Appendix 2). At the same time, this interaction effect was a tendency for post-grazing DM mass, plantain leaves, sulfur, sodium, chloride and catalpol ($P < 0.1$) (Table 3.3). The effect of pasture treatment on the main measured experimental parameters in different seasons is presented in Figure 3.2. Overall, the trend of treatment effect was consistent over seasons; however, the effect level on those variables differed between seasons. Specifically, the treatment effect on the plantain reproductive stem in early summer was greater than in spring, late summer, and autumn. The higher composition of plantain stem resulted in a lower content of plantain leaves in early summer compared with other seasons. In addition, the stronger effect of pasture treatment on dead materials, DM%, OMD, and sodium was observed in late summer and autumn than in spring and early summer.

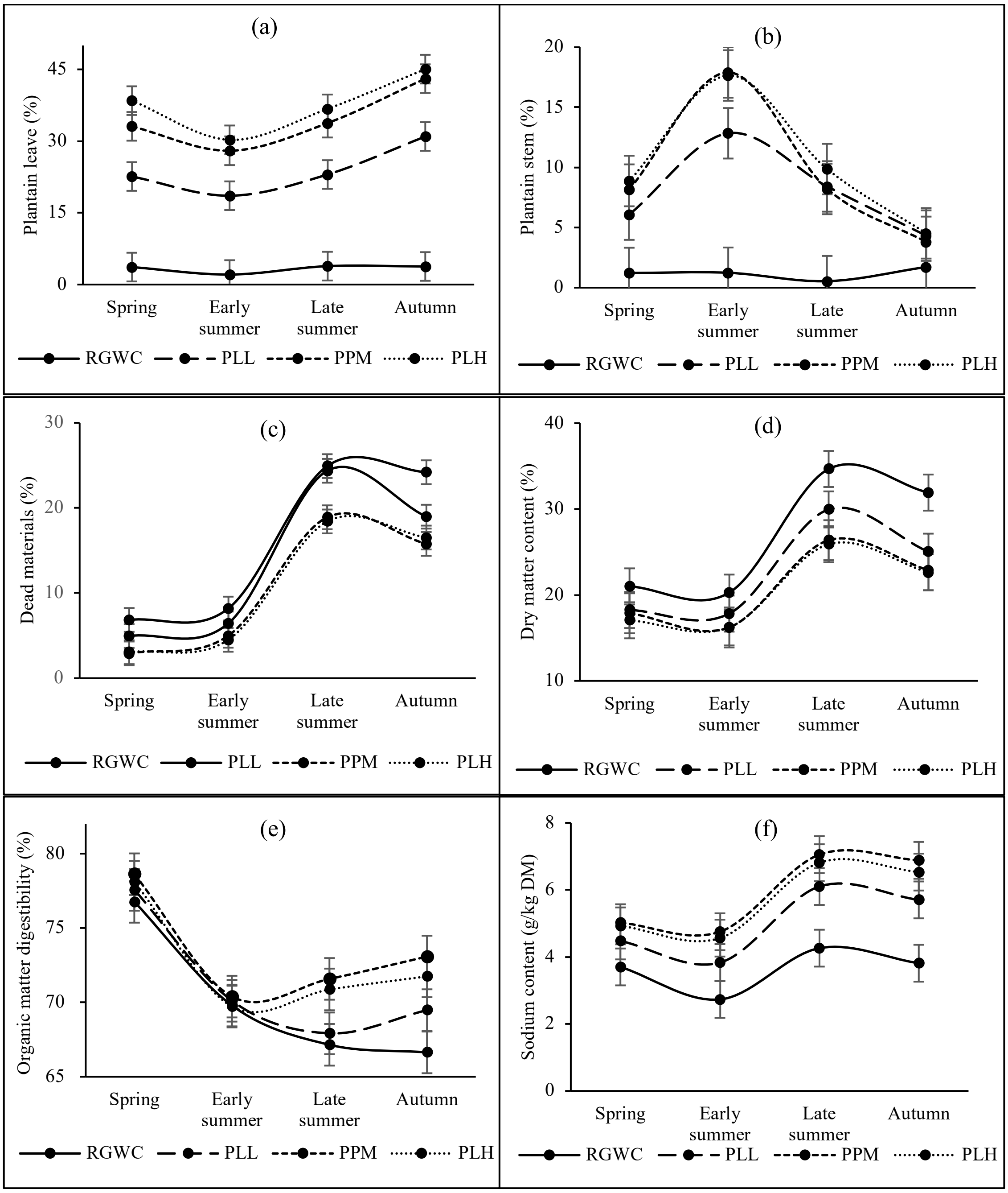

Figure 3.2. Composition of plantain leaves (a), plantain reproductive stem (b), dead materials (c), dry matter content (d), organic matter digestibility (e), and sodium (f) of pasture treatments in different seasons over the experimental period.

Note: RGWC = perennial ryegrass-white clover, PLL = RGWC + low plantain rate, PLM = RGWC + medium plantain rate, PLH = RGWC + high plantain rate.

3.5. Effect of plantain content and season

The relationships between the content of plantain leaves in the pasture and herbage nutritive value traits in different seasons are presented with general linear regression models covariate with the season in Table 3.5.

Table 3.5. Relationship between the content of plantain leaves and parameters related to botanical composition, nutritive value, mineral content, and bioactive compound in different seasons over the experimental period.

Variable	α	β	Spring	Early summer	Late summer	Autumn	$P_{plantain}$	P_{season}	R^2	r.m.s.e
Ryegrass (%)	81	−1.03	0^a	-18.2^b	-31.3^c	-17.1^a	<0.001	<0.001	0.75	12.2
Dead (%)	6.9	−0.10	0^a	1.1^a	17.2^b	15.1^b	<0.001	<0.001	0.75	4.2
DM (%)	21	−0.11	0^a	-1.4^a	10.7^b	7.7^c	<0.001	<0.001	0.60	3.9
OMD (g/kg DM)	757	0.73	0^a	-71^b	-81^b	-77^b	<0.001	<0.001	0.51	41
ADF (g/kg DM)	255	−0.46	0^a	32^b	22^c	16^c	<0.001	<0.001	0.42	19
NDF (g/kg DM)	436	−1.61	0^a	59^b	38^c	36^c	<0.001	<0.001	0.59	34
Ash (g/kg DM)	108	0.28	0	−3.0	−3.7	2.6	<0.001	0.100	0.20	13
CF (g/kg DM)	31	0.06	0^a	-4.9^b	-3.4^c	-3.2^c	<0.001	<0.001	0.36	2.9
NSC (g/kg DM)	235	0.93	0^a	-49^b	-12^b	-29^b	<0.001	<0.001	0.28	44
Phosphorus (g/kg DM)	4.0	0.01	0^a	-0.02^a	-0.42^b	-1.08^c	<0.001	<0.001	0.53	0.4
Sulfur (g/kg DM)	2.4	0.02	0^a	0.43^b	0.53^b	0.38^b	<0.001	<0.001	0.49	0.4
Calcium (g/kg DM)	7.3	0.15	0^a	1.0^a	2.7^b	1.3^a	<0.001	<0.001	0.64	2.2
Magnesium (g/kg DM)	2.1	0.01	0^a	0.12^b	0.81^c	0.98^d	<0.001	<0.001	0.73	0.28
Sodium (g/kg DM)	3.0	0.06	0^a	-0.14^a	1.67^b	0.98^c	<0.001	<0.001	0.51	1.3
Chloride (g/kg DM)	11.2	0.21	0^a	-3.0^b	-0.80^a	0.88^a	<0.001	<0.001	0.51	4.3
Iron (mg/kg DM)	198	−1.22	0^a	62^b	-14^a	54^b	<0.001	<0.001	0.16	92
Manganese (mg/kg DM)	110	−0.45	0^a	16^b	0.4^a	27^c	<0.001	<0.001	0.22	26
Zinc (mg/kg DM)	26	0.29	0^a	10^b	12^{bc}	14^c	<0.001	<0.001	0.61	6.3
Copper (mg/kg DM)	10.3	0.06	0^a	1.9^b	2.6^b	1.2^b	<0.001	<0.001	0.18	2.0
Boron (mg/kg DM)	11.6	0.18	0^a	0.2^a	4.0^b	-1.4^a	<0.001	<0.001	0.37	3.8
Aucubin (mg/g DM)	−0.68	0.18	NA	0	0.79	0.10	<0.001	0.186	0.70	2.1
Acteoside (mg/g DM)	1.27	0.16	NA	0^a	1.68^b	-1.0^a	<0.001	0.001	0.47	3.1
Catalpol (mg/g DM)	0.51	0.01	NA	0^a	-0.12^a	-0.47^b	<0.001	<0.001	0.28	0.43

Note: $^{a, b, c, d}$ values within a row with different superscripts differ at $P < 0.05$ between seasons; R^2 = the coefficient of determination; r.m.s.e = root mean square error. NA = not applicable (variables were not measured in spring). The model: $y = \alpha + \beta \times$ plantain content (%) + season; where y = independent variable, α = intercept, β = slope of plantain effect (% plantain leaves), season = spring (September-November), early summer (December), late summer (January-February), Autumn (March-May).

Plantain leaves percentage significantly influenced all the given parameters in Table 3.5. Specifically, the proportion of plantain leaves was negatively associated with the composition of perennial ryegrass and dead materials, DM content, ADF, NDF, iron, and manganese ($P < 0.05$). In contrast, it was positively associated with OMD, ash, CF, NSC, phosphorus, sulfur, calcium, magnesium, sodium, chloride, zinc, boron, catalpol, aucubin, and acteoside ($P < 0.05$). In addition, the effect of season was significant on all given parameters in Table 3.5, except for ash and aucubin ($P < 0.05$). In particular, ryegrass, OMD, CF and NSC contents across pasture treatments were higher in spring than in other seasons. At the same time, the composition of dead materials, DM%, ADF, NDF, and most minerals (sulfur, calcium, magnesium, sodium, zinc, copper, and boron) was higher in late summer and autumn than in spring ($P < 0.05$). The R^2 of the model was higher than 0.50 for the content of ryegrass, dead materials, DM%, OMD, NDF, phosphorus, calcium, magnesium, sodium, chloride, zinc, and aucubin, was between 0.3 and 0.5 for ADF, CF, sulfur, boron, and acteoside, and was lower than 0.3 for ash, NSC, iron, manganese, copper, and catalpol.

3.4. Discussion

The current study confirmed that under a dairy grazing system, plantain-based pasture has a similar annual yield to RGWC (Al-Marashdeh et al., 2021). Additional analyses from the present study found no difference in the DM yield between the plantain-based pastures and the RGWC pasture in any season and no statistical relationship between seasonal yield with plantain rate and climate factors such as rainfall, water deficit, and soil temperature. A previous grazing study indicated that annual and seasonal DM yields are maintained at the farm scale with a plantain-based pasture compared to RGWC pasture (Al-Marashdeh et al., 2021). Other studies reported a higher DM yield of mixed pastures containing plantain than the RGWC pasture. However, those mixtures included more pasture species such as chicory *(Cichorium intybus)*, prairie grass *(Sporobolus cryptandrus)*, lucerne *(Medicago sativa)*, and red clover *(Trifolium pratense)* (Nobilly et al., 2013; Pembleton et al., 2015; Woodward et al., 2013). Perhaps, the variation in the pasture DM yield of the paddock-scale measurement is considerably large (Stephen & Revfeim, 1971); therefore, a big difference between treatments may be required to demonstrate significance. The results of the present study suggest that under a rain-fed grazing dairy system, incorporating 32–48% plantain into RGWC pasture maintains the annual DM yield of the pasture in the first two years from sowing.

The biggest challenge for farmers is to maintain a high proportion of plantain in the RGWC-based pasture, especially under grazing conditions (Dodd et al., 2019). The plantain content in the present study was greater than in newly established mixed pastures with multiple forage species (Bryant et al., 2018; Totty et al., 2013; Woodward et al., 2013) or when plantain seed was added into an existing RGWC sward (Bryant et al., 2019). However, it was lower than in mixed pastures with plantain and clover (Al-Marashdeh et al., 2021; Rodriguez et al., 2020). The low plantain composition in PLH indicated that incorporation of plantain into RGWC pasture is unlikely to achieve 70% or more plantain for an extended period under grazing conditions. Plantain seeds added by direct drilling at the end of the first year did not successfully increase plantain content in the pastures. In the present study, the establishment of young plantain was not observed as they could not compete well with the existing swards. A similar result has been indicated in a study by Bryant et al. (2019). The failure of adding plantain seed suggests that the proportion of plantain in the present experiment was obtained from the original establishment. In addition, this result agrees with Dodd et al. (2019) and Bryant et al. (2019) that the plantain percentage in grass-based pastures increases in the first year and then declines in the second year from establishment. The time to reach the highest plantain content in the current study (421–455 days) is relatively similar to Bryant et al. (2019), who indicated the highest plantain content was achieved between 432 and 470 days after sowing. In the present study, the pasture sown with a high plantain seed rate (PLH) reached the highest plantain content earlier than those with the lower rate (PLM), but then it declined faster to have a similar plantain composition as PLM in the second year. Plantain has been reported to have a high DM yield early after the autumn sowing (Powell et al., 2007). Over time, plantain continually disappears due to its short lifespan and the competition from stronger ryegrass and white clover in the mixed pasture (Dodd et al., 2019; Tiley & France, 1990). This plantain loss may occur faster in the pasture with a higher ryegrass density; for example, amongst treatments, PLM and PLH were better than PLL in achieving and maintaining a high plantain content over grazing events. A survey of 21 New Zealand farms (Dodd et al., 2019) showed that most ryegrass and clover-based mixed paddocks had less than 20% plantain three years after sowing. This low plantain content is not expected to significantly improve farm productivity and environmental benefit [32]. Therefore, further studies on interventions and managements are required to maintain plantain content in mixed swards beyond two years from the establishment.

The main advantages of plantain-based pasture include an improvement in herbage nutritive value, potentially increasing animal performance and reducing N losses from the dairy system (Condren et al., 2019). The current study is consistent with previous studies that included plantain in RGWC pasture resulting in increased NSC (Minneé et al., 2019), the content of minerals (Mangwe et al., 2019), and bioactive compounds (Box et al., 2017), but decreased fibre content (McCarthy et al., 2020). Previous studies have reported that pure plantain had a lower DM content by 30% and NDF by 33%, and contained a higher NSC by 30%, and total mineral content by 26%, compared to ryegrass (Minneé et al., 2019). Plantain was also reported to contain 0.44–6.87 g/kg DM of aucubin and 0.5–41.7 g/kg DM of acteoside (Navarrete et al., 2016). Those inherent differences cause changes in herbage nutritive characteristics when plantain is incorporated into RGWC pasture in the current experiment. The comprehensive data set in the present study found a significant difference between the plantain-based pasture and the RGWC pasture in parameters with a smaller effect, such as lignin, OMD, starch, CF, sulfur, magnesium, iron, manganese, zinc, copper, boron and cobalt. These parameters have either not been reported or were inconsistently or not significantly affected by plantain inclusion in previous studies (Box et al., 2017; Mangwe et al., 2019; Navarrete et al., 2016). Those changes in nutritional value have been reported to improve animal performance and provide environmental benefits. Specifically, the lower fibre content of plantain can increase DM intake because animals can consume and digest more plantain-based pasture than RGWC pasture in the same period, thus increasing milk production (Nguyen et al., 2022). In addition, the greater content of moisture and minerals can result in an increase in water intake and urine volume to dilute UN of animals (O'Connell et al., 2016). Moreover, bioactive compounds, aucubin, and acteoside in plantain can reduce ammonia production in the rumen to decrease UN excretion of grazing animals, and the extraction of these compounds from roots and leaves can inhibit nitrification processes in soils to decrease NO_3^- leaching and nitrous oxide emission from pastoral systems.

Regarding the effect of pasture treatment, there is a clear trend that the pastures sown with more plantain seed had a greater difference in herbage nutritive characteristics than RGWC. A certain amount of plantain content is required to have a measurable improvement in the value of several nutritive parameters. For example, the concentration of OMD, ADF, lignin, ash, starch, CF, magnesium, manganese, and cobalt of plantain-based pasture significantly differed from RGWC when containing 44% plantain or more (PLM and PLH) but were not statistically different when containing 32% plantain (PLL). In support of this, previous studies (Minnée et al., 2020; Nkomboni et al., 2021) concluded that the RGWC-based pasture requires more than 30% plantain to cause a measurable effect on reducing UN excretion, while improving herbage nutritive value and, hence, milk production of dairy cows. The greater impact of the plantain-based pastures in late summer and autumn on key parameters (DM%, OMD, sulfur, sodium, copper, and cobalt) aligns with the hypothesis that plantain improves herbage quality during drier seasons (Metson & Saunders, 1978; Navarrete et al., 2016; Tamura, 2001). The greater improvement in the pasture quality in late summer and autumn is expected to expand the effect of plantain-based pastures on animal performance and environmental benefits during the drier months when the nutritive value of ryegrass is lower (P.D. Kemp, 2000). The results of the current research suggest that under a grazing dairy system, the plantain-based pastures sown with 7 kg (PLM) and 10 kg (PLH) plantain seed are better than those sown with 4 kg (PLL) plantain seed in improving nutritive value in the first two years from sowing.

The impact of plantain incorporation on pasture quality may be influenced by plantain content and differ across seasons. The present study generated four models to estimate the relationship between each nutritive parameter and the content of plantain leaves in spring, early summer, late summer, and autumn. The high coefficient of determination of the estimated models for DM content, OMD, NDF, phosphorus, calcium, magnesium, sodium, chloride, zinc, and aucubin ($R^2 > 0.5$) resulted from a significant difference between plantain and RGWC in these nutritive characteristics (Mangwe et al., 2019; Minneé et al., 2019; Navarrete et al., 2016). For the seasonal effect, the result of the current study was consistent with other studies that plantain-based pasture contains a higher DM content, ADF, NDF, and most minerals and has a lower concentration of OMD, CF, and NSC in late summer and autumn than in spring (Minneé et al., 2019; Navarrete et al., 2016; Stewart, 1996; Tamura, 2001). The concentration of minerals has been suggested to be affected by precipitation and is usually higher in dry summer and autumn (Gershenzon, 1984; Stamp & Deane Bowers, 1994). The present study has not considered other factors potentially influencing herbage nutritive value, such as grazing intervals, leaf age, water stress, and temperature (Stamp & Deane Bowers, 1994; Stewart, 1996; Tamura, 2001). These may be the reasons for a low R^2 ($R^2 < 0.3$) of the estimated models for several parameters (lignin, ash, NSC, iron, manganese, copper, and catalpol). Further studies are needed to investigate the effect of these associated factors on the quality of pastures containing plantain.

The role of plantain on dairy farms to reduce N losses is currently highly topical. Plantain can either be sown as a monoculture crop, incorporated with conventional pastures at sowing to form a mixed sward, or added into existing pastures by broadcasting or direct drilling (Bryant et al., 2019; Dodd et al., 2019). However, there is a limited understanding of the effectiveness of these establishment methods on plantain content, DM yield, and herbage nutritive value. Bryant et al. 2019 indicated that direct drilling could achieve a higher plantain rate than broadcasting for adding plantain into an existing RGWC pasture; however, these two methods rarely reach more than 30% of plantain in the sward (Bryant et al., 2019). Therefore, the new establishment of plantain-based pasture could allow farmers to obtain a higher plantain content in the cow diet in the first two years. From the current study, 30% of plantain in a dairy farm might be achieved if the whole farm was sown with plantain equivalent to the PLL treatment rate (see sowing rates in Table 3.1), or if about 60% of the farm area was sown with plantain equivalent to the PLM or PLH treatment rate. However, the proportion of plantain in the swards will be maintained at

30% or more for only the first 24 months from establishment. Pure plantain or plantain-clover has been reported to achieve higher plantain content than plantain-based pasture (Dodd et al., 2019). However, the pure plantain pasture has been found to have various disadvantages such as poor winter growth, sensitivity to treading damage, weed ingression, risk of bloat to the animal, and low palatability in the seeding season (Stewart, 1996). Further studies are required to investigate suitable methods to get plantain into the dairy system and to sustain plantain content in the plantain-based pasture beyond two years after the establishment.

3.5. Conclusions

The present study indicated that incorporating plantain with RGWC with a sowing rate between 4 and 10 kg plantain seed per hectare maintains an annual DM yield close to the RGWC pasture in the first two years after sowing. Over two years, the average composition of plantain across the plantain-based pasture treatments was 41%. The percentage of plantain in the plantain-based pastures increased in the first year to reach a peak of 46 and 64% at 421 and 455 days, then declined to 20–30% plantain at day 705 after sowing. In conclusion, incorporating plantain into RGWC pasture is unlikely to achieve 70% or more plantain under grazing conditions over an extended period. Moreover, maintaining more than 30% plantain in the pasture-based pasture after two years will likely require a management intervention such as sowing in more plantain.

In addition, incorporating plantain with RGWC pasture resulted in a lower concentration of DM, ADF, NDF, CF, iron, and manganese but a higher concentration of OMD, ash, starch, NSC, phosphorus, sulfur, magnesium, sodium, chloride, zinc, copper, boron, cobalt, aucubin, acteoside, and catalpol in comparison to the RGWC pasture. Furthermore, the impact of pasture treatment on dead materials, DM content, OMD and sodium was greater in late summer and autumn than in spring and early summer. Moreover, the proportion of plantain leaves was strongly associated with decreased concentration of dead materials, DM, OMD, NDF, and increased concentration of phosphorus, calcium, sodium, magnesium, chloride, zinc, and aucubin in the pastures.

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CHAPTER 4

IMPACT OF PLANTAIN-BASED PASTURE ON URINE VOLUME AND URINATION FREQUENCY OF GRAZING DAIRY COWS

Chapter 3 indicated that plantain incorporation increased water and mineral contents of the pasture, potentially affecting animal urine production. This chapter determined the impact of grazing plantain-based pasture on the urine volume and urinary frequency of dairy cows. The results of this chapter will provide evidence to link the effect of the plantain incorporation on NO_3^- leaching from pastoral systems.

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Simple Summary

Incorporating forage plantain into the diet of grazing dairy cows has the potential to reduce nitrogen (N) losses to the environment. One of the fundamental mechanisms for reduced N loss from plantain-fed cows is increased urine volume and dilute urine N concentration, increasing the percentage of urine N used for plant growth. The present study aimed to determine whether lesser proportions of plantain in the diet would effectively achieve changes in urination. The results showed that compared to cows grazing a perennial ryegrass-white clover pasture, cows with 25% plantain in their apparent intake diet increased urine volume and urination frequency and reduced urine N concentration in the morning. When dietary plantain was less than 20%, there was only a small effect on increased urine volume, though morning urine N concentration was reduced. Ensuring more than 20% plantain in the diet has the potential to reduce N leaching risk via reduced urine N load from grazing dairy cows.

Abstract

The objective of the present study was to determine the effect of grazing plantain-based pasture on urine volume, urination frequency, and urine nitrogen (UN) concentration of dairy cows under a typical pastoral dairy practice offering approximately 25% supplemented feeds. The experiment was a completely randomised design with three pasture treatments: perennial ryegrass-white clover (RGWC), RGWC + low plantain rate (PLL), and RGWC + high plantain rate (PLH), five replicate plots and repeated in two sequential grazing periods. Forty-five lactating Friesian × Jersey cows were randomly assigned into three groups of 15 animals each to graze over 6 days in adaptation paddocks and 3 days in experimental plots. Urine flow sensors were used to measure urine volume and urinary frequency, while spot urine sampling was conducted to determine nitrogen (N) concentration in cow urine. The results showed that including 25% plantain in the diet (PLH) increased daily urine volume by 44% and the daily number of urinations by 28%, compared to grazing the RGWC pasture. In addition, the N concentration in cow urine was decreased by 18 and 29% when the diet contained 18% and 25% plantain, respectively. In conclusion, under a typical dairy farm practice, incorporating plantain into RGWC pasture with the proportion of 25% plantain in the diet can increase the number of urine patches and reduce the concentration of N in the urine, thereby providing the opportunity to decrease N leaching from pastoral systems.

Keywords: *Plantago lanceolata,* nitrogen excretion, urination frequency, urination behaviour, urine volume.

4.1. Introduction

Nitrogen (N) excreted in cow urine is the primary source of N losses from pastoral dairy systems worldwide (Humphreys, 2008). Dairy cows grazing traditional swards deposit onto pastoral soils in small localised urine patches with an N rate between 200 to 2000 kg N/ha on a wetted area between 0.14–0.49 m^2 per urination (Selbie et al., 2015). Ideally, the N in soils is used by microbes and retained in organic form or by pastures in mineral form. The average N load in urine patches usually exceeds the N requirement of pastures, resulting in the risk of nitrate (NO_3^-) leaching and nitrous oxide emissions (Cameron et al., 2013; Di & Cameron, 2002). On average, only 41% of urine N (UN) can be taken up by pastures, while 20% of UN could be lost as NO_3^- leaching from pastoral systems (Selbie et al., 2015). Regulations have been developed to limit the amount of N loss from intensive dairy farms into the environment to improve water quality (Minister for Housing Planning Local Government and Heritage, 2022; Minister for the Environment, 2020). Therefore, dairy farmers are required to incorporate strategies to manage their farms and meet the regulations on N losses. Mitigation strategies often target reducing the concentration of N in the urine of cows to reduce the amount of UN excreted into soils by dairy cows (Di & Cameron, 2002).

Plantain (*Plantago lanceolata*) has been increasingly used in pastoral systems to overcome feed deficits of the conventional perennial ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) (RGWC) pasture and improve milk production of dairy cows, especially during the dry summer due to its tolerance to drought and heat (Dodd et al., 2019). In recent years, plantain has emerged as a forage pasture with attributes that reduce N losses from pastoral dairy systems (MPI, 2021). Particularly, the risk of UN being leached is high in late summer and autumn when the herbage growth rate is low, causing more NO_3^- being accumulated in soils (Navarrete et al., 2022). Research has consistently confirmed the role of plantain in reducing N concentration in cow urine (Navarrete et al., 2022; Nguyen et al., 2022a) and altering the urination behaviours of dairy cows (Mangwe et al., 2019; Minnée et al., 2020). In addition, these changes are influenced by plantain proportion in the diet in that a certain composition of plantain is required to have a measurable effect (Minnée et al., 2020; Nkomboni et al., 2021). In practice, farmers are challenged to achieve sufficient quantities of plantain in the pasture. Furthermore, in autumn, when the effect of plantain on reducing N loss is important, the content of plantain in the diet is further diluted by the use of supplements to offset the decline in pasture growth.

There is limited research on the impact of different proportions of plantain in grazed diets. Most existing studies have either been conducted with feeding cut pastures indoors (Minnée et al., 2020) or grazing swards without supplements (Dodd et al., 2018; Mangwe et al., 2019). Nkomboni et al. (2021) showed that increasing plantain content from 20–70% reduced U N concentration in spot samples of grazing cows in late lactation. However, because NO_3^- leaching risk is driven by N load from urine, information is required about both N concentration and urine volume (Li et al., 2012; Romera et al., 2012). The effect of different proportions of plantain on N load risk has not been elucidated in previous research. In practice, achieving consistently high proportions of plantain (>20%) in the diet can be challenging, particularly when feeding supplements. Previous research has shown that less than 15% plantain in the diet is unlikely to affect urination behaviours (Bryant et al., 2018; Minnée et al., 2020). The study of Minnée et al. (2020) was carried out under cut and carry conditions, and the study of Nkomboni et al. (2021) measured urine N% but not urine volume. More information is required regarding the effect of low to moderate plantain proportion on the urination behaviour of grazing dairy cows.

Farmer adoption of alternative feeds for mitigating N loss risk requires information on both environmental and production outcomes in order to assess the impact on their farm system and build confidence. The objective of the present study is to determine the effect of the RGWC-based pasture containing sizeable percentages of plantain under a typical grazing practice on milk production and urination behaviour of late lactation dairy cows.

4.2. Materials and Methods

The experiment was conducted over two sequential grazing periods, between the 23–31 March 2021 and between 5–13 May 2021, at Massey University No. 4 Dairy Farm, Palmerston North, New Zealand (40°23'27"S 175°36'44"E), according to the experimental procedure approved by Massey University Animal Ethics Committee (MUAEC #21/09). The study site is on Tokomaru silt loam soil (Hart et al., 1988) and has a temperate climate. The detailed soil and climate condition, pasture establishment and management are described in Chapter 3.

4.2.1. Experimental Design and Management

The experiment was a completely randomised design with three pasture treatments, five replicate plots (800 m^2/plot), and two repeated grazing periods (09 days/period). Pasture treatments were RGWC (perennial ryegrass cv. ONE^{50} and white clover cv. Tribute), RGWC + low plantain (cv. Agritonic) rate (PLL), and RGWC + high plantain rate (PLH). The swards were established in April 2019 by direct drill and rotationally grazed by dairy cows, with nine grazing events yearly. The sowing rates were designed to have low and high rates of plantain using seed rates where plantain seed was approximately 20% or 35% of the sowing rate and reflected options farmers might use in practice. The sowing rates of plantain, perennial ryegrass and white clover at sowing were 0, 20, and 3 kg in RGWC, 4, 15, and 3 kg in PLL, and 7, 10, and 3 kg in PLH, respectively. Urea fertiliser (46% N) was applied at 90 kg N/ha/year, with the latest application at 30 kg N/ha on 20th November 2020. The pasture was mown on 13th February 2021 to a consistent height of 6 cm before the study commenced in March 2021. After each defoliation event in February 2021 and March 2021, the pasture was monitored (using a rising plate meter) to achieve a compressed height of 16–18 cm (approx. 2,800 kg dry matter (DM) kg/ha) to start the experimental periods.

Forty-five Jersey-Friesian lactating cows were selected from 100 approved cows and assigned to their pasture treatments in each grazing period. Group balance was based on milk yield (19.0 ± 3.4 kg/day in March 2021 and 17.1 ± 3,9 kg/day in May 2021), days in milk (216 ± 13 days in March 2021 and 240 ± 13 days in May 2021), live weight (566 ± 29 kg in March 2021 and 554 ± 43 in May 2021), and somatic cell count (95,000 ± 20,100 in March 2021, and 335,000 ± 61,000 in May 2021) (mean ± SE). Approved cows grazed the same RGWC pasture and were managed under a similar farm practice as in the present research before the experimental periods. The data used for group balance were obtained from the milking system three days before each experimental period (milk yield, day in milk and live weight) and from a herd test conducted a week before the experiment periods (somatic cell count). In each nine-day grazing period, cows were adapted to their treatment pastures over the first 6 days by strip grazing together in their mobs of 15 cows (approx. 130 m^2/cow/day) using temporary fencing materials. Then, each group of 15 cows was randomly subdivided each day into five replicated mobs of 3 cows/mob and allocated for grazing over 3 days in experimental plots (3 cows per each 800

m^2 plot). At the end of the first grazing period, cows were returned to the farm, and another 45 cows were selected to use in the second grazing period.

During the experimental periods, cows were managed under a typical farm practice, milked twice daily at 07:00 h and 15:00 h. Pasture and supplement allocation to meet energy requirements resulted in approximately 16 kg DM pasture diet above a target post-grazing residual of 1,500 kg DM/ha and five kg DM of supplement. The pasture area allocated for each day was estimated by a rising plate meter and adjusted every half-day by visually assessing the post-grazing residual. For instance, the area was increased when the residual was below the target and vice versa. Supplements were fed in a trough on a concrete feed pad once daily after morning milking by feeding all experimental cows at once for over two hours. Supplement composition and group intake were not determined during the study as all cows were fed together in a single group, though utilisation was over 90%. The supplement was provided with 3 kg maize silage, 1 kg corn gluten pellets and 1 kg pasture baleage. The chemical composition of the supplement was not measured; however, typical maize silage, corn gluten pellets and pasture baleage in New Zealand have respective DM content of 33, 89 and 38%, ME content of 10.3, 12.7 and 9 MJ/kg DM, and CP of 8, 23 and 15% (Dairy NZ, 2021). After finishing the supplemented feeds, the cows were sent to graze their pastures. Cows spent approximately 18 hours grazing in the paddocks and six hours milking and eating supplements. Fresh water was available *ad libitum* from troughs in each treatment plot and at the feed pad. Minerals were added (per cow/day) at 40 g salt coarse (containing grade 22 NaCl), 40 g MgO (54% Mg), 05 g AquaTrace (10 mg cobalt, 150 mg copper, 04 mg iodine, 04 mg selenium and 60 mg zinc) in drinking water through an inline dispenser.

4.2.2. Herbage measurements

Pastures were measured for each plot as a replication to determine the herbage DM intake, botanical composition, and nutritive value. Herbage DM mass was measured by randomly harvesting three quadrat cuts to ground level (0.1 m^2) a day before cows started grazing in the plots for pre-grazing and were repeated a day after the grazing for post-grazing. The samples were cleaned by manually removing soil and faecal debris and oven-dried at 75 ^oC until a constant weight was achieved. Herbage DM intake was estimated for each group of cows grazing in individual plots via the equation: DM intake (kg DM/cow/day) = (pre-grazing mass (kg DM) - post-grazing mass (kg DM)) ÷ (no. of cows × no. of grazing days). Trough water intake was calculated with a similar procedure using the data recorded by a flow meter (Gardena Water Smart Flow Meter) attached to a water trough in each plot. Nutritive value and botanical composition of the pasture were measured with a hand-plucked sample to the grazing height (approx. 7 cm). The sample was taken with 15–20 grabs (approx. 400g fresh weight) by zigzag pattern across each plot at 10:00 h a day before cows started grazing in the plots. Each sample was placed in a plastic bag and stored at 4^oC in a cold room to prevent water evaporation before being weighed. Each sample was mixed thoroughly and subsampled into two smaller samples. The first sub-sample (approx. 100g fresh weight) was manually separated into plantain leaves, plantain reproductive stem and seed head, ryegrass, white clover, weeds, and dead materials. Then, it was oven-dried at 75 ^oC until a constant weight was achieved to calculate the botanical composition of each component. The second sub-sample was recorded for fresh weight and oven-dried at 60 ^oC until a constant weight was achieved to determine the DM content of the pastures. Then, the dried samples were ground to pass a 1mm sieve for chemical analysis. The analyses were conducted by a commercial laboratory (Hill Laboratory, Hamilton, New Zealand), using near infra-red spectroscopy method, for OM, crude fat (CF), crude protein (CP) (N × 6.25), acid detergent fibre (ADF), neutral detergent fibre (NDF), non-structural carbohydrates (NSC) (NSC = 100-(CP + ash + CF + NDF), OM digestibility (OMD), metabolisable energy (ME) (calculated from OMD), sodium, potassium, calcium and sulfur (nitric acid/hydrogen peroxide digestion) (Hill Lab, 2021). In addition, plant bioactive compounds: aucubin and acteoside were analysed with high-performance liquid chromatography at Massey University using the method described by Navarrete et al. (2016).

4.2.3. Animal measurements

Milk yield was recorded for each cow twice daily during the morning milking at 07:00 h and the afternoon milking at 15:00 h with an automatic system (Waikato Milking System). In addition, a milk sample (approx. 30 ml) was collected from each cow during morning and afternoon milking on day seven of the experimental periods. Milk samples were analysed for total solids (vacuum oven, AOAC 990.19, 990.20), protein content (Dumas method for N, P = 6.25 × N), and milk urea nitrogen (MUN) (Urease Kinetic UV assay). Urine and faecal spot samples were collected from each cow after morning milking at 09:00 h on days seven and eight of the experimental periods. Urine samples (approx. 80 ml each) were taken by vulva stimulation and sub-sampled into two smaller samples (approx. 30 ml each). The first subsamples were acidified with sulfuric acid 6.0 normal to reduce pH to below 4.0 and analysed for N concentration (Dumas method). The second sub-samples were analysed for the content of creatinine (Jaffe method), sodium, potassium, and chloride (ion-selective electrode method). Faecal samples were taken by rectal stimulation and recorded for fresh weight. Then, these samples were freeze-dried until a constant weight was achieved to estimate DM content. The dried samples were ground to analyse for N concentration (Dumas method) and non-protein N (colourimetric method). All milk, urine and faecal samples were stored at -20 °C until analysed. The analyses of these samples were conducted by a certified laboratory (Nutrition Laboratory, Massey University).

4.2.4. Urine volume and urination frequency

Daily urine volume, urination volume and the number of urinations were measured using Lincoln University PEETER V1.0 urine flow sensors (Marshall, Beck, Garrett, Beale, et al., 2021). In each grazing period, sixteen cows were selected from the 45 experimental cows to wear urine sensors over two runs of 36-h for a 24-h measurement (8 cows per run). In the first run, eight urine sensors were attached to the vulva of eight cows (as shown in Figure 4.1) on day six following the morning milking and were removed following the afternoon milking on day seven. In the second run, sensors were attached to eight cows on day seven after the afternoon milking and removed on day nine following the morning milking. After milking, cows were moved to a vet race where the sensors were attached, a process which took about 45 minutes and allowed cows to become accustomed to wearing the sensor. The number of cows wearing sensors was balanced in each run with 2 or 3 cows per group, resulting in 11 cows in RGWC, 10 cows in PPL and 11 cows in PPH for both grazing periods. Sensor attachment was monitored as cows walked between the paddock and the milking shed and in the milking parlour at each milking. Sensors which became detached and were leaking urine were immediately re-attached or recorded for data exclusion.

Figure 4.1. PEETER V1.0 urine sensors attached to cows during the experiment.

4.2.5. Statistical Calculations and Analysis

Variables of herbage (DM mass, botanical composition and nutritive value), animal (milk, urine and faecal samples), and sensor (urine volume and the number of urinations) were analysed using PROC mixed procedure of SAS (SAS Institute, 2020) according to the model: $Y_{ijk} = m + T_i + T_i(G_j) + C_k + e_{ijk}$, where Y_{ijk} = dependent variable; m = overall mean; T_i = fixed effect of pasture treatment I; $T_i(G_j)$ = fixed effect of treatment i nested within grazing period j; C_k = random effect (plot for herbage variables and apparent intake or cow for animal and sensor variables), and e_{ijk} = residual error. Comparisons of means were analysed using Fisher's Least Significant Difference test. A significant difference was declared at $P < 0.05$, and a tendency was declared at $P < 0.10$.

The N concentration in the urine was estimated as the mean of spot samples collected from individual cows over two measurement days. Nutritive value traits, botanical composition of the pastures, herbage DM intake and water intake at the paddock (from herbage intake and water troughs) were analysed with five replicate plots per treatment in each grazing period (n=30, 5 plots × 3 treatments × 2 grazing periods). In addition, variables measured with animal spot samples (milk, urine, and faeces) were analysed with the replicated individual cows (n = 90, 15 cows × 3 treatments × 2 grazing periods).

The data on urine volume and urination frequency from the sensors were scanned to either exclude or include from the dataset. A sensor was eligible when it recorded data for at least 24 hours. The 24 hours was determined from the second urination to mitigate the effect of sensor attachment on the first urination. Event data were excluded when the time from the prior urination was less than five minutes, the event duration was less than five seconds, or events had a similar curve and volume as the previous one (Mangwe et al., 2019; Shepherd et al., 2017). The data from six sensors were excluded from the dataset because it was recorded for less than 24 hours (4) or sensors were noted as leaking urine (2). As a result, 26 cows wearing sensors were eligible for urine volume and urination frequency, with ten in RGWC, eight in PLL, and eight in PLH. There was a total of 651 urinations recorded over 36 hours while the cows wore sensors, in which 409 urination events occurred during 24 measurement hours.

4.3. Results

The average daily temperature and rainfall during the regrowth and grazing periods are presented in Table 4.1. In general, the weather during the regrowth and grazing periods in March 2021 was hotter and drier than in May 2021. In addition, sunrise and sunset occurred between 07:36 and 19:20 h during the grazing period in March 2021 and 07:22 h and 17:17 h during the grazing period in May 2021.

Table 4.1. Average temperature and the total rainfall during the regrowth and grazing periods.

Variable	Average temperature (^{o}C)	Total rainfall (mm)
Regrowth period before March grazing	17.1	1.9
Grazing period in March grazing	17.1	1.2
Regrowth period before May grazing	14.4	2.9
Grazing period in May grazing	15.3	1.6

Note: climate data was achieved from a climate station 1 km from the experimental site.

4.3.1. Herbage characteristics

Herbage DM mass, botanical composition, nutritive value, mineral content, and bioactive compounds of the pastures over two grazing periods are presented in Table 4.2. Pre-grazing DM mass was similar between treatments (2,788 ± 253 kg DM/ha) ($P > 0.10$). However, the herbage residual of PLH and PLM tended to be lower than in RGWC ($P < 0.10$). Cows were allocated an estimated 30 kg DM to ground level from the pastures with an area of 130 m^2 per cow per day.

Table 4.2. Herbage mass, botanical composition, and nutritive value of pasture treatments.

Variable	RGWC	PLL	PLH	SEM	P
Herbage mass					
Pre-grazing mass (kg DM/ha)	2,815	2,784	2,745	62	0.725
Post-grazing mass (kg DM/ha)	1,551	1,425	1,358	61	0.096
Allocation area (m^2/cow/d)	130	130	130	-	-
DM allocation (kg /cow/d)	30.0	29.7	29.3	0.66	0.701
Botanical composition					
Plantain (g/kg DM)	-	243^a	342^b	17	<0.001
Perennial ryegrass (g/kg DM)	611^a	380^b	305^c	21	<0.001
White clover (g/kg DM)	236	266	249	16	0.409
Dead material (g/kg DM)	145^a	107^b	103^b	7.9	0.001
Nutritive value					
DM content (%)	20.4^a	17.6^b	16.3^c	0.25	<0.001
OM (%)	86.8	85.6	84.9	0.33	0.004
CF (g/kg DM)	35^a	29^b	29^b	0.7	<0.001
CP (g/kg DM)	231	232	234	5.1	0.889
ADF (g/kg DM)	268	255	254	4.3	0.060
NDF (g/kg DM)	484^a	441^b	424^b	8.0	<0.001
NSC (g/kg DM)	124^a	154^b	162^b	8.0	0.008
OMD (g/kg DM)	719	718	722	7.8	0.919
ME (MJ/kg DM)	10.0	9.8	9.8	0.12	0.418
Sodium (g/kg DM)	4.9^a	5.9^b	6.5^b	0.31	0.004
Potassium (g/kg DM)	26^a	$28a^b$	30^c	0.90	0.011
Calcium (g/kg DM)	8.4^a	14.0^b	15.8^c	0.52	<0.001
Sulfur (g/kg DM)	13^a	17^b	19^b	0.7	<0.001
Aucubin (g/kg DM)	0.18^a	2.68^b	4.58^c	0.34	<0.001
Acteoside (g/kg DM)	0.29^a	6.77^b	10.86^c	0.71	<0.001

Note: $^{a, b, c}$ Mean values in the same row with different superscripts differ ($P < 0.05$). ADF = acid detergent fibre, CF = crude fat, CP = crude protein, DM = dry matter, ME = metabolisable energy, NDF neutral detergent fibre, NSC = non-structural carbohydrates, OM = organic matter, OMD = OM digestibility, RGWC = perennial ryegrass-white clover, PLL = RGWC + low plantain rate, PLH = RGWC + high plantain rate, SEM = standard error of the means.

The proportion of plantain was 243 g/kg DM in PLL and 342 g/kg DM in PLH ($P < 0.05$), in which reproductive stem and seed head accounted for less than 10 g/kg DM in all treatments. The proportion of perennial ryegrass and dead materials in PLL and PLH was lower than in RGWC ($P < 0.05$). There was no difference in white clover content between treatments ($P > 0.10$). Compared to RGWC, PLL and PLH had a lower composition of DM (%), OM, CF and NDF but contained a higher composition of NSC, potassium, sodium, calcium, and sulfur ($P < 0.05$). The higher plantain content in PLH resulted in this treatment having a lower DM content and higher potassium, calcium, aucubin and acteoside than PLL ($P < 0.05$).

4.3.2. Milk production

The apparent herbage intake of cows was similar between treatments, averaging 15.0 ± 2.5 kg DM/cow/day ($P > 0.10$). Together with supplemented feed, the total apparent intake was 19.5 ± 2.5 kg DM/cow/day ($P > 0.10$). Plantain intake was 3.5 kg DM in PLL, and 4.9 kg DM in PLH, accounting for 18 ± 2.1 and 25 ± 2.6% of the total diet ($P < 0.01$).

There was no difference in daily milk yield, total milk solids (content and yield), and milk protein (content and yield) between cows grazing RGWC, PLL and PLH (Table 4.3, $P > 0.10$). Over two grazing periods, cows produced an average of 17.6 ± 2.9 kg milk, 2.54 ± 0.40 kg total milk solids, and 0.69 ± 0.13 kg milk protein. Milk urea N was the only milk component affected by pasture treatment, with MUN in PLH and PLL 22% and 12% lower than in RGWC, respectively ($P < 0.05$).

Table 4.3. Herbage intake, milk yield and total milk solids, milk protein, and milk urea N of cows grazing different pasture treatments.

Variable	RGWC	PLL	PLH	SEM	P
Herbage intake (kg DM/cow/day)	14.1	15.2	15.4	0.65	0.329
Total apparent intake (kg DM/cow/day)[1]	18.7	19.6	19.8	0.77	0.546
Plantain intake (kg DM/cow/day)	0.0[a]	3.5[b]	4.9[c]	0.32	<0.001
Plantain content of the diet (%)	0.0[a]	17.7[b]	24.6[c]	1.45	<0.001
Milk yield (kg/cow/day)	18.1	17.6	17.4	0.52	0.577
Milk solids (g/100 g milk)	14.4	14.8	14.3	0.21	0.231
Milk solids (kg/cow/day)[2]	2.63	2.61	2.47	0.07	0.178
Milk protein (g/100 g milk)	3.9	4.1	3.9	0.07	0.135
Milk protein (kg/cow/day)	0.71	0.71	0.66	0.02	0.104
Milk urea N (mg/dL)	13.8[a]	12.1[b]	10.8[c]	0.25	<0.001

Note: [a, b, c] mean values in the same row with different superscripts differ ($P < 0.05$); [1] assuming cows utilised 90% of provided supplemented feeds; milk solids = fat, protein, lactose and ash; RGWC = perennial ryegrass-white clover; PLL = RGWC + low plantain rate; PLH = RGWC + high plantain rate.

4.3.3. Urine and faecal characteristics

Urine volume, urination frequency, urine and faecal characteristics, and water intake of dairy cows grazing different pastures are presented in Table 4.4. Cows grazing the pastures with low (PLL) and high (PLH) plantain rates urinated 20% and 44% more total daily urine volume than grazing the RGWC pasture ($P < 0.05$). The increased volume was achieved through an increased number of events, as the average volume per urination did not differ between treatments. The concentration of N in the urine of cows grazing PLH and PLL was 29% and 18% lower than when grazing RGWC ($P < 0.05$). Urine creatinine and faecal N were not different between treatments ($P > 0.10$). Regarding mineral content in the urine, cows grazing the plantain-based pastures (PLL and PLH) had a higher chloride, similar potassium, and lower sodium than when grazing the RGWC pasture ($P < 0.05$).

Water intake from the herbage diet was greater in cows grazing PLH and PLL than those grazing RGWC. Cows partially compensated by consuming less trough water when more water was consumed in the herbage ($P < 0.05$). Compared to RGWC, grazing PPL and PLH resulted in an 22 and 29L/cow/day increase in herbage water intake and a 7 and 10L/cow/day decrease in water intake from drinking troughs, respectively. As a result, the water intake from herbage and water troughs at the paddock of cows grazing PLL and PLH was 15 and 19 L more than that of cows grazing RGWC ($P < 0.05$). Regarding water output, cows grazing PLH and PLL excreted more water in urine ($P < 0.05$) but had a lower water content in faeces ($P < 0.05$).

Table 4.4. Urine volume, urination frequency, urine and faecal characteristics, and water intake of dairy cows grazing pasture treatments.

Variable	**RGWC**	**PLL**	**PLH**	**SEM**	**P**
Apparent herbage N intake	507	539	560	26.8	0.392
Urine volume and urination pattern					
Urine volume (L/cow/day)[1]	40.8^{a}	49.1^{ab}	58.9^{b}	3.66	0.008
Urination (urinations/cow/day)[1]	13.3	14.8	17.0	1.05	0.064
Urination volume (L/urination)[1]	3.0	3.5	3.5	0.22	0.187
Urinary N (g/kg)[2]	5.5^{a}	4.5^{b}	3.9^{b}	0.23	<0.001
Urine creatinine (mmol/L)[2]	2,406	2,140	2,270	106	0.199
Urine urea (mmol/L)[2]	242^{a}	197^{b}	172^{b}	7.0	<0.001
Urine NH_3 (mmol/L)[2]	2.5	2.8	2.7	0.15	0.427
Urine Na (mmol/L)[2]	88^{a}	75^{b}	72^{b}	3.84	0.008
Urine K (mmol/L)[2]	169	176	176	4.6	0.457
Urine Cl (mmol/L)[2]	121^{a}	171^{b}	181^{b}	6.8	<0.001
Faecal characteristic					
Faecal DM content (%)[2]	11.3^{a}	12.6^{b}	13.6^{c}	0.34	<0.001
Faecal N (g/kg DM)[2]	27.7	28.1	27.4	0.38	0.485
Water intake					
Herbage water intake (L/cow/day)[3]	57^{a}	79^{b}	86^{b}	3.6	<0.001
Trough water intake (L/cow/day)[3]	23^{a}	16^{b}	13^{b}	1.5	<0.001
Total apparent water intake (L/cow/d)[4]	85^{a}	101^{b}	105^{b}	4.0	0.015

Note: $^{a, b, c}$ Mean values in the same row with different superscripts differ ($P < 0.05$). [1] calculated with the data from cows wore urine sensors (n = 26) with 10 in RGWC, 8 in PLL and 8 in PLH. [2] calculated with all cows (n = 90). [3] calculated with the data from pasture plots (n = 30). [4] assuming cows had similar water from supplements of 5.5 L/cow/day, estimated with the supplement DM% of 45% and the utilisation of 90%, RGWC = perennial ryegrass-white clover, PLL = RGWC + low plantain rate, PLH = RGWC + high plantain rate.

4.4. Discussion

The results of the present study indicate that under the farm practice offering both grazed pastures and supplemented feeds, grazing the plantain-based pastures increased volume and urination frequency while reducing N concentration in the urine. In addition, increasing plantain content up to 25% of a supplemented pasture diet did not affect milk yield, milk solids, or protein in milk.

The milk yield of dairy cows fed with a diet containing plantain has been reported to either maintain or increase, depending on the stage of lactation and the difference in DM intake (DMI) and energy intake between experimental and control diets (Minneé et al., 2017; Nguyen et al., 2022a; Nkomboni et al., 2021). The higher DMI and ME intake can improve energy and protein balance to increase milk production when the diets have a similar protein intake (Combellas & Hodgson, 1979). The current results are consistent with previous studies, where DMI and ME content were similar between the plantain-based pastures and the RGWC pasture, resulting in an equivalent milk yield and milk solids of late lactation dairy cows. Furthermore, several studies have reported an increased milk protein yield of dairy cows by including plantain in the diet. This is due to the higher ME and undegraded N content in plantain-based pasture than in RGWC pasture, which is suggested to improve the utilisation of N by partitioning more N to milk rather than to urine (Minnée et al., 2020; Wilson et al., 2020). The present study showed no difference in ME content and herbage N intake between the plantain-based pastures and the RGWC pasture. Although undegraded N intake was not measured, there may not have been much difference between pasture treatments when the diet contained 25% plantain or less. Consequently, grazing the plantain-based pastures did not affect milk protein yield in the present study.

In the current research, dairy cows grazing pastures containing plantain had increased urine volume, which is most likely driven by the difference in DM content between the plantain-based pastures and the RGWC pasture. Specifically, the higher water content in plantain compared with RGWC can increase the water intake of dairy cows (Mangwe et al., 2019; Minnée et al., 2020), resulting in more water excreted to urine if other water outputs are not affected (Khelil-Arfa et al., 2012; Shepherd et al., 2017). In the present study, 18 and 25% dietary plantain increased herbage water intake by 22 and 29L/cow/day. The higher herbage water intake reduced drinking water from the troughs but increased the total apparent water intake at the paddock by 16 and 20L/cow/day. At the same time, the water excreted to milk was similar between treatments, while the water excreted to faeces of the cows grazing the plantain-based pastures might be lower than when grazing RGWC due to the lower faecal DM content. Therefore, the excessive water input is likely excreted into the urine, increasing urine output by 9 and 18L/cow/day. A similar effect of water intake on urine output was observed when comparing the data between two grazing periods. The lower herbage DM content in May 2021 compared with that in March 2021 resulted in 34 L more in the water intake from herbage and water troughs at the paddock and 27 L more in the urine volume of cows. The available facility only allowed us to measure water intake from herbage and water troughs from the experimental plots, but not the water intake during supplementation at the barn. Therefore, further studies with more urine sensors and the entire water balance are required to estimate the relationship between water intake and urine volume and urination frequency of dairy cows that are known to affect N losses from pastoral systems (Di & Cameron, 2007; Marsden et al., 2016).

The increase in urine volume in the farm condition of the present study was observed when cows grazed a diet containing 25% plantain or consumed 4.9 kg DM plantain per day. However, urinary creatinine from spot samples was not different between cows grazing the plantain-based pastures and the RGWC pasture. Differently, in a longer-term measurement period using more cows, urine creatinine of cows grazing the same plantain-based pastures (PLL and PLH) was lower than that in the RGWC pasture (Chapter 5). These results suggest that the creatinine method may underestimate the effect of plantain-based pasture on urine volume when the diet contains a low percentage of plantain, the sample size is small, and spot samples were collected once daily. The diurnal changes of urine creatine require a strong effect (by a high proportion of plantain), more sampling points or possibly more replicate cows to have a representative estimation of urine volume from urine creatinine (Lee et al., 2019). In practice, models can be developed to estimate the impact of plantain-based pasture on urination parameters with a lower percentage of plantain in the diet when sufficient input data are achieved (Kebreab et al., 2009; Romera et al., 2012).

Although the current study measured urination behaviour for a relatively short period, the study agrees with previous research regarding the effect of plantain on increasing urine volume (Mangwe et al., 2019; Minnée et al., 2020). Similarly, the results of the present study align with previous research that a greater urine production is associated with increased urination frequency and reduced N concentration in the urine rather than the volume of each urination when plantain is included in the diet. The 20 and 44% increase in urine volume and 18 and 29% reduction in UN concentration suggest that UN excretion was probably similar between all treatments. This result aligns with previous studies that at least 30% plantain in the diet is required to have a measurable effect on reducing UN excretion by more than 10% (Minnée et al., 2020; Navarrete et al., 2022). Although the mechanisms for reducing UN excretion are not fully understood, the lower UN excretion from plantain-fed cows with similar N intake as control animals are generally associated with greater partitioning of N to dung and milk (Marshall, Beck, Garrett, Barrell, et al., 2021; Minnée et al., 2020). This is through a combination of improved rumen synchrony of energy and protein (improvement in milk N) and lower protein degradability (greater faecal N). The high NSC content and bioactive compounds acteoside and aucubin in plantain have been reported to influence rumen fermentation and microbial N production to improve N utilisation (Marshall, Beck, Garrett, Barrell, et al., 2021; Navarrete et al., 2016).

When the total amount of UN excretion is maintained, the dilution of UN is, in part, a key driver from plantain-based pasture to reduce NO_3^- leaching at the paddock level. The implications of these results can be realised when considering urine patch area and N load. Using the equation in Romera et al. (2012) (Appendix 3), the area affected by urine patches from a single animal (m^2) can be estimated. Based on the volumes in Table 4.4, the area affected by urine is 4.1, 4.9 and 5.9 m^2/cow/day for RGWC, PLL and PLH, respectively. Assuming the lower UN concentration of plantain-fed cows is consistent across all urinations, the N loads under the affected area were 553, 470, and 403 kg N/ha equivalent (estimated using Equation as in Appendix 4). Previous lysimeter research with varying N loads from 0 to 800 kg N/ha has demonstrated a positive relationship between N load and nitrate (NO_3^-) leaching (Ledgard et al., 2015; Romera et al., 2012). In addition, plantain inhibits the nitrification processes in the soil (Carlton et al., 2019; Podolyan et al., 2019). While incorporating plantain into the RGWC pasture can maintain milk production (as in the present study), and pasture production (as in Chapter 3 and by Nguyen et al. (2022b)), its effect on increasing the number of urine patches and reducing UN concentration could provide a successful low-cost option to reduce N leaching from pastoral systems. The increased urine volume and frequency can distribute a similar UN into a larger area with a lower N load. As a result, pastures have the opportunity to uptake more N from urine patches, resulting in a lower risk of N losses from pastoral systems (Di & Cameron, 2002; Woods et al., 2018). Using the APSIM model, Ledgard et al. (2015) indicated that the decrease of 40% in UN concentration with a similar UN excretion (by increasing urine volume and urination frequency) led to a reduction in NO_3^- leaching by 50% from urine patches, and 22% from grazing paddocks.

4.5. Conclusions

In a typical grazing practice with dairy cows, grazing the pasture containing 25% plantain in the diet increased urine volume by 44% and the number of urine patches by 28%. In addition, 18 and 25% of dietary plantain reduced N concentration in cow urine by 18 and 29%. The ability of pastures containing less than 25% plantain to reduce NO_3^- leaching from pastoral systems is likely associated with increasing urine volume and urination frequency rather than decreasing UN excretion. Further studies are necessary to investigate the effect of plantain-based pasture on NO_3^- leaching at the paddock scale and the effective mechanisms before adopting this technology on a wider scale.

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CHAPTER 5

IMPACT OF PLANTAIN-BASED PASTURE ON MILK PRODUCTION AND NITROGEN EXCRETION OF GRAZING DAIRY COWS

Chapter 5 measures the impact of grazing the plantain-based pasture on milk production and urine nitrogen excretion of dairy cows. The results of this chapter and those from Chapter 3 will demonstrate the benefits of plantain incorporation into the RGWC pasture on farm productivity, and those from Chapter 4 will provide evidence for the effect of plantain incorporation on nitrate leaching.

A paper from this chapter is in preparation to submit to Sustainability – MDPI as:

Nguyen, T.T.; Navarrete, S.; Horne, D.; Donaghy, D.; Kemp, P. Milk production and nitrogen excretion of grazed dairy cows in response to plantain content and lactation season.

Abstract

Plantain proportion in mixed pastures and the quality of plantain-based pastures varies in different seasons, potentially causing different effect on milk production and nitrogen (N) excretion of grazing. The objective of the present study was to quantify milk yield and composition and urine nitrogen (UN) excretion of dairy cows grazing pasture containing increasing rates of plantain under a typical farm practice. Pasture treatments were perennial ryegrass (RGWC), RGWC + low plantain rate (PLL), RGWC + medium plantain rate (PLM) and RGWC + high plantain rate (PLH). Experimental pastures were established in April 2019 and grazed by cows over 14 grazing during two lactation years between September 2019 and March 2021. The results showed no significant difference between pasture treatments in milk production, the yield and composition of milk solids, protein, fat, and lactose. However, cows grazing pastures containing an average of 23% dietary plantain had lower UN concentration by 22%, lesser UN excretion by 7%, and higher urine volume by 33%, compared with cows grazing RGWC pasture. Furthermore, the changes in UN concentration, UN excretion and urine volume were associated with the percentage of plantain in the diet and were greater in late summer and autumn than in spring and early summer. These results suggest that plantain-based pasture can reduce the risk of nitrogen losses from pastoral systems and have no negative effect on milk production for at least two lactation years from the establishment.

5.1. Introduction

Dairy production from grazed pastures in temperate countries such as New Zealand and Ireland mainly relies on perennial ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) (RGWC) pasture (Kemp, 1999). This pasture type can persist well under grazing conditions, providing high economic performance for dairy farms (Verkerk, 2003). However, the RGWC pasture has been known to produce low quality and quantity of herbage in hot and dry conditions, thus often causing feed deficits during summer. In addition, its high nitrogen (N) content often exceeds the N requirement of livestock, resulting in the risk of N losses from pastoral systems (Cameron et al., 2013). In recent decades, governments have developed regulations to reduce the environmental impact of agricultural production (Minister for Housing Planning Local Government and Heritage, 2022; Minister for the Environment, 2020). These legislations have raised interest in developing management practices to limit N leaching from dairy systems.

Plantain *(Plantago lanceolata)* is a forage herbage tolerant to drought and heat (Stewart, 1996). In recent decades, plantain has increasingly been used worldwide in grazing swards to mitigate environmental impact and improve farm productivity of dairy farms (Doole et al., 2021). Previous studies have indicated that including plantain in the diet can increase milk yield, milk solids and protein in milk, and decrease urine nitrogen (UN) excretion by dairy cows (McCarthy et al., 2020; Nguyen et al., 2022a). Furthermore, the differences in UN concentration, urine volume and UN excretion of dairy cows could be associated with plantain composition in the diet (Minnée et al., 2020; Nkomboni et al., 2021) and varied in different seasons (Box et al., 2017; Dodd et al., 2018). However, these studies have been conducted in short periods, either with cut pastures fed indoors (Minnée et al., 2020; Minneé et al., 2017) or by grazing pastures without supplements (Box et al., 2017; Nkomboni et al., 2021). The effect of plantain in these conditions may differ from the actual effect under typical farm practices that commonly offer cows both grazing pastures and supplemented feeds.

The advantages of plantain-based pasture are expected to provide farmers with a low-cost option to reduce N losses from dairy systems to meet regulatory requirements. For instance, research at the paddock scale demonstrated that grazing plantain pasture could halve the N leaching from pastoral dairy systems over an entire lactation season while sustaining milk solids production compared to grazing RGWC pasture (Navarrete Quijada et al., 2019).

However, it is important to determine and maintain adequate proportions of plantain to obtain the environmental benefit for an extended period when established with RGWC in grazing systems (Dodd et al., 2019; Nguyen et al., 2022b). In addition, the composition of plantain and the quality of its herbage vary significantly in different seasons and with the maturity of pastures (Bryant, Dodd, et al., 2019), leading to a variable effect on milk production and the UN excretion of dairy cows (Box et al., 2017; Navarrete et al., 2022). Therefore, before adopting this technology widely on farms, farmers need to have clear evidence of the effect of incorporating plantain with RGWC pasture on milk production and UN excretion of dairy cows under typical farm practices for extended periods. The results from Chapter 3 of the thesis have indicated that plantain content when incorporated with RGWC under grazed conditions, increases in the first 1.5 years after sowing before declining rapidly in the next 1.5 years (Nguyen et al., 2022b). Moreover, plantain content in pastures is positively associated with non-structural carbohydrates (NSC), the content of minerals, and bioactive compounds and is negatively related to dry matter (DM) and fibre content. Changes in plantain content and nutritive value may affect milk production and UN excretion of dairy cows over seasons.

The purpose of the present study was to evaluate the effects of grazing the pasture incorporated with increasing content of plantain on milk yield and milk composition and the UN excretion of dairy cows under a typical farm practice over an extended period of two lactation years. In addition, the impact of plantain incorporation on milk production and UN excretion was examined in association with dietary plantain content and lactation seasons.

5.2. Materials and Methods

The study was conducted over two lactation seasons from September 2019 to March 2021 (2019/2020 and 2020/2021 lactation years) at Massey University's No 4 Dairy Farm, Palmerston North, New Zealand (40°23'26.9" S 175°36'43.5" E). The experimental procedures were approved by Massey University Animal Ethics Committee (MUAEC#19/54). The study site was on Tokomaru silt loam soil with a temperate climate. The specific soil test and climate information over the experimental period were described in Chapter 3.

5.2.1. Experimental design and management

Pasture treatments were four mixed swards established with increased plantain (cv. Agritonic) seed rates: RGWC RGWC (perennial ryegrass cv. ONE^{50} and white clover cv. Tribute), RGWC + low plantain rate (PPL), RGWC + medium plantain rate (PLM), and RGWC + high plantain rate (PLH). A 7 ha experimental site was divided into four adaptation areas of 1 ha and 20 experimental plots of 800 m^2, then randomly assigned to be sown with pasture treatments. The pastures were established on April 5th, 2019, by direct drilling per ha with 20 kg ryegrass + 3 kg clover for RGWC, 4 kg plantain + 15 kg ryegrass + 3 kg clover for PLL, 7 kg plantain + 10 kg ryegrass + 3 kg clover for PLM, and 10 kg plantain + 5 kg ryegrass + 3 kg clover for PLH. Urea fertiliser (46% N) was applied at 120 kg N/ha annually during the study period. The detailed establishment and management of the experimental swards are described in Chapter 3 and by Nguyen et al. (2022b).

The pastures were grazed by dairy cows at 4–6-week intervals, resulting in 14 grazing events over the experimental period. In each grazing period (8–9 days), 60 or 80 Jersey-Friesian dairy cows were assigned to their pasture treatments according to the balance in milk yield, days in milk, and body weight. Cows were adapted to their pastures over the first 6 days by strip grazing (approx. 100 or 130 m^2/cow/day) using temporary electric fences. Then, each group of 15 or 20 cows were randomly assigned for grazing in five treatment plots over a 1.5–3-day measurement period (3 or 4 cows per plot). Cows were managed under a typical farm practice, milked twice daily at 07:00 h and 15:00 h, and allocated 14–20 kg DM of pasture and 5–8 kg

DM of their diet as concentrated supplements (maize silage, corn gluten pellet, and pasture silage).

Table 5.1. The summary of animals, feeds, and measurements during grazing events over the experimental period.

Grazing period	**No. cows**	**Milk yield (L/day)** (mean ± SD)	**Plantain leaves (% in pasture)** (PLL:PLM:PLH)	**Herbage intake (kg DM/day)** (mean ± SD)	**Supplement offered** (kg DM/day)	**Measurement**
2019/2020						
12–20/09	80	35.4 ± 5.4	12:21:35	17.4 ± 2.2	7.5	MY, MC
26–24/10	80	32.8 ± 5.4	11:19:28	18.8 ± 4.4	7.5	MY
13–21/11	80	31.6 ± 4.7	15:22:31	14.0 ± 2.3	7.5	MY
14–12/12	80	30.7 ± 4.2	18:29:30	13.8 ± 2.2	6.0	MY, MC, NE
9–17/1	80	26.5 ± 3.3	28:31:39	17.8 ± 2.6	6.0	MY
5–12/2	60	23.5 ± 3.4	28:37:41	18.3 ± 4.8	6.0	MY, MC, NE
2–19/3	60	17.4 ± 2.9	40:57:61	15.8 ± 3.9	7.5	MY, MC, NE
2020/2021						
27/8–5/9	60	38.8 ± 5.5	42:63:61	14.1 ± 3.0	8.0	MY, MC
7–15/10	80	33.2 ± 4.7	31:47:46	16.7 ± 2.4	6.5	MY, MC
11–19/11	80	29.3 ± 4.7	23:28:30	14.3 ± 1.9	8.0	MY, MC
2–10/12	60	27.5 ± 3.6	17:29:30	18.5 ± 2.5	8.0	MY, MC, NE
6–14/1	80	24.6 ± 3.4	11:23:22	13.9 ± 2.0	5.0	MY
3–10/2	60	23.1 ± 3.3	18:31:33	17.2 ± 2.9	5.0	MY, MC, NE
24–31/3	60	18.2 ± 2.0	21:30:29	16.2 ± 2.7	5.0	MY, MC, NE

Note: MC = milk composition, MY = milk yield, NE = nitrogen excretion, PLL = perennial ryegrass and white clover (RGWC) + low plantain rate, PLM = RGWC + medium plantain rate, PLH = RGWC + high plantain rate, SD = standard deviation, DM = dry matter.

The pasture area allocated for each day was estimated with a rising plate meter and adjusted every half-day by visually estimating the post-grazing residual. Supplements were fed in a trough on a concrete feed pad twice daily after morning milking and before afternoon milking. Supplement composition and intake were not determined during the study as all cows were fed together in a single group, though utilisation was over 90%. Approximately 80% of supplements were maize silage, corn gluten pellets, pasture silage and dried distillers grains. Maize silage, corn gluten pellets, pasture silage, and dried distillers grains in New Zealand have respective DM content of 33, 89, 38 and 90%, metabolisable energy (ME) of 10.3, 12.7, 9.0 and 13 MJ/kg DM, and crude protein (CP) of 8.0, 23, 15 and 28% (Dairy NZ, 2021). Fresh water was available *ad libitum* from troughs in each treatment plot and at the feed pad. On average, cows spent approximately 18 hours grazing in the paddock and 6 hours milking and feeding on supplements at the shed. The main characteristics of the animals, feeds, and management during grazing events over the study period are described in Table 5.1.

5.2.2. Herbage measurements

Herbage measurements were conducted for individual plots to determine DM mass, DM intake (DMI), and botanical and chemical compositions. Herbage DM mass and DMI were measured for all 14 grazing over the experimental period by harvesting three quadrat cuts to ground level (0.1 m^2) a day before grazing for pre-grazing mass and repeated a day after grazing for post-grazing mass. The samples were oven-dried at 75 ^oC until a constant weight was achieved. Apparent herbage DMI was estimated for a group of 3 cows grazing in individual plots via the equation: DMI (kg DM/cow/day) = (pre-grazing mass kg DM - post-grazing mass kg DM) ÷ (no. of cows × no. of grazing days).

Botanical and chemical compositions were measured for the ten grazings that coincided with animal measurements (Sep 2019, Dec 2019, Feb 2020, Mar 2020, Sep 2020, Oct 2020, Nov 2020, Dec 2020, Feb 2021, Mar 2021). A hand-plucked sample was taken by randomly collecting 15–20 grabs to grazing height from each plot at 10:00 h a day before grazing. Each sample was mixed thoroughly and divided into two subsamples. The first subsamples (approx. 100g fresh weight) were manually separated into plantain leaves, plantain stem and seed head, perennial ryegrass, white clover, weeds, and dead materials. These components were then oven-dried at 75 ^oC until a constant weight was achieved to calculate botanical composition. The second subsamples were weighed for fresh weight and then oven-dried at 60 ^oC until a stable weight was achieved to determine DM content. These samples were ground to pass through a 1 mm sieve for chemical analysis. The analyses were conducted by a commercial laboratory (Hill laboratory, Hamilton, New Zealand), using near infra-red spectroscopy, for CP (N × 6.25), crude fat (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), NSC, organic matter digestibility (OMD), ME, chloride, potassium, calcium, sodium (nitric acid/hydrogen peroxide digestion). The samples were also analysed for aucubin and acteoside by high-performance liquid chromatography at Massey University according to the method described in Navarrete et al. (2016).

5.2.3. Animal measurements

Animal measurements were conducted to determine milk yield and composition, urine, and faecal characteristics and to estimate urine volume, UN and faecal N (FN) excretions of dairy cows. Milk yield was measured for individual cows in all 14 grazings by an automated system (https://waikatomilking.com/). Milk solids, milk protein and milk urea nitrogen (MUN) were evaluated in four grazings during the lactation year 2019/2020 (Sep 2019, Dec 2019, Feb 2020, Mar 2020) and in six grazings during the lactation year 2020/2021 (Sep 2020, Oct 2020, Nov 2020, Dec 2020, Feb 2021, Mar 2021). In these grazings, milk samples (approx. 30 ml) were collected for individual cows during morning milking (07:00 h) and afternoon milking (15:30 h) on day one and day seven of the grazing periods. The milk samples from eight grazings (Sep 2019, Dec 2019, Feb 2020, Mar 2020, Sep 2020, Dec 2020, Feb 2021, March 2021) were analysed for milk solids (vacuum oven, AOAC 990.19, 990.20), protein content (Dumas method for N, protein = 6.25 × N), and MUN (Urease Kinetic UV assay), by a certified laboratory (Nutrition Laboratory, Massey University, Palmerston North, New Zealand). Milk fat and lactose were assessed in six grazings in the lactation year 2020/2021 (Sep 2020, Oct 2020, Nov 2020, Dec 2020, Feb 2021, Mar 2021). Replicated samples (approx. 50 ml) were taken from individual cows during morning milk and afternoon milking on day one and day seven of these six grazings. The samples were analysed for milk solids, the content of protein, fat, lactose, and MUN using a MilkoScan FT1 (FOSS, Hilleroed, Denmark). The duplicate data from the certified laboratory analyses in three grazings (Dec 2020, Feb 2021, and Mar 2021) were used to calibrate MilkoScan FT1 data. Calibration equations were developed using the PROC reg procedure of SAS (SAS Institute, 2020), with a coefficient of determination value (R^2) of 0.78 for MUN and > 0.91 for other parameters (Appendix 6). Consequently, the dataset of milk solids, protein and MUN included the data from eight grazings analysed by the certified laboratory (Sep 2019, Dec 2019, Feb 2020, Mar 2020, Sep 2020, Dec 2020, Feb 2021, March 2021), and two grazings analysed by MilkoScan FT1 (Oct 2020 and Nov 2020). At the same time, the milk fat and milk lactose dataset were the calibrated data of MilkoScan FT1 from six grazings in the lactation year 2020/2021.

The characteristics of urine and dung were measured for each cow in three grazings during the late lactation period of each experimental year (Dec 2019, Feb 2020, March 2020, and Dec 2020, Feb 2021, and Mar 2021). Urine and faecal samples were collected after morning milking on days 7 and 8 of the grazing period. Urine samples (approx. 80 ml each) were taken by vulva stimulation and subsampled into two smaller samples (approx. 30 ml each). The first subsample was acidified with sulfuric acid (6.0 normal) to reduce pH to below 4.0 and analysed for N concentration (Dumas method). The second subsample was analysed for the content of creatinine (Jaffe method), sodium, potassium and chloride (ion-selective electrode method). Faecal samples were taken by rectal stimulation and recorded for fresh weight. Then, these samples were freeze-dried until a constant weight was achieved to estimate the DM content. The dried samples were ground through a 1 mm sieve and then analysed for N concentration (Dumas method) and non-protein N (NPN) (colourimetric method). The analyses of urine and faecal samples were conducted by a certified laboratory (Nutrition Laboratory, Massey

University, Palmerston North, New Zealand). Urine volume and the amount of UN and FN excretions were estimated according to the equations below:

- Urine volume (kg/day) = 21.9 × body weight (kg) × (1 ÷ urinary creatinine (mg/kg)) (Pacheco et al., 2007).
- UN excretion (g/day) = urine volume (kg) × UN (g/kg) (Pacheco et al., 2007).
- FN excretion (g/ day) = FN (g/kg) × DMI (kg/day) × (1 - digestibility (%)) (Ahvenjärvi et al., 2018).

5.2.4. Data interpreting and statistical analysis

The analyses for milk, urine and faecal variables were conducted using the PROC mixed procedure of SAS according to the model: $Y_{ijkl} = \mu + T_i + S_j + Y_k + (T \times S)_{ij} + (T \times Y)_{ik} + (S \times Y)_{jk} + (T \times S \times Y)_{ijk} + \beta_1 I_l + C_l + e_{ijkl}$, where: Y_{ijkl} = dependent variable; μ = overall mean; T_i = treatment i; S_j = season j (spring = September to November, early summer = December to January, late summer = February, Autumn = March); Y_k = year k; $(T \times S)_{ij}$ = the interaction between treatment i and season j; $(T \times Y)_{ik}$ = the interaction between treatment i and year k; $(S \times Y)_{jk}$ = the interaction between season j and year k; $(T \times S \times Y)_{ijk}$ = three-way interaction between treatment i, season j and year k; β_1 = the slope of the linear effect of covariance with I_l; I_l = initial data for milk variables (data measured on day 1 of the grazing period), or body weight for urine, plasma and faecal variables of cow l; C_l = random effect of cow l; the e_{ijkl} = residual error. The analysis for pasture variables used the same model with the random effect of plot number instead of cow number and by removing the covariance factor. A significant difference between means was declared at $P < 0.05$.

In addition, the relative change in UN concentration, urine volume, MUN, and UN excretion, FN excretion and N partitioning to milk was analysed to determine the relationship with the percentage of plantain leaves in the diet. The analysis was conducted using the PROC corr procedure in SAS (SAS Institute, 2020). The plantain content in the diet was calculated for the mean of treatment in each grazing. Relative change in a variable was estimated according to the equation: RC = 1 - Y_{PLi}/Y_{RGWC}, where Y_{PLi} and Y_{RGWC} refer to the mean value of cows grazing treatment i and RGWC in a grazing, respectively. A significant correlation between the two variables was declared at $P < 0.05$. The linear regression between the relative change in UN concentration and urine volume with the percentage of plantain leaves in the diet was determined using PROC reg in SAS.

5.3. Results

5.3.1. Herbage characteristics

The botanical and chemical compositions of the pastures over the experimental period are shown in Table 5.2 and Appendix 5. All the variables were affected by treatments ($P < 0.05$) except white clover, weeds, and CP content. The composition of plantain leaves, plantain stem and seed head, ryegrass, white clover, and dead materials were 33, 12, 620, 164, and 160 g/kg DM for RGWC; 238, 79, 388, 145, and 137 g/kg DM for PLL; 345, 95, 289, 157, and 106 g/kg DM for PLM; and 376, 102, 260, 144, and 106 g/kg DM for PLH, respectively. Weeds accounted for less than 13 g/kg DM for all treatments.

For chemical composition, on average, the pastures containing plantain (PLL, PLM and PLH) had a higher content of NSC by 19%, OMD by 4%, Ca by 62, Na by 57%, and Cl by 29%, but contained a lower DM content by 21%, along with CF by 5%, ADF by 5%, and NDF by 11%, compared to RGWC. In addition, the plantain-based pastures contained an average of 4.8 g/kg DM aucubin and 8.2 g/kg DM acteoside. There was no significant difference in CP, ME, and K content between treatments. Pre-grazing mass was similar between treatments; however, post-grazing mass in PLM (1,770 kg DM/ha) and PLH (1,860 kg DM/ha) were lower than in RGWC (2,030 kg DM/ha) ($P < 0.05$). On average, cows across treatments had a herbage intake of 16.5 kg DM/day ($P > 0.05$).

Table 5.2. Botanical and chemical compositions of pasture treatments over the experimental period.

Variable	RGWC	PLL	PLM	PLH	SEM	P_{T}	$P_{T×S}$	$P_{T×Y}$	$P_{T×S×Y}$
Pre-grazing mass (1,000 kg DM/ha)	3.56	3.51	3.35	3.52	0.09	0.348	0.989	0.925	0.944
Post-grazing mass (1,000 kg DM/ha)	2.03^{a}	1.93^{ab}	1.77^{b}	1.86^{b}	0.07	0.043	0.380	0.073	0.350
Plantain leaves (g/kg DM)	33^{a}	238^{b}	345^{c}	376^{c}	12.9	<0.001	0.145	0.243	<0.001
Plantain stem (g/kg DM)	12^{a}	79^{b}	95^{b}	102^{b}	9.4	<0.001	0.008	0.347	0.761
Total plantain (g/kg DM)	45^{a}	317^{b}	440^{c}	478^{c}	16.9	<0.001	0.77	0.023	<0.001
Ryegrass (g/kg DM)	620^{a}	388^{b}	289^{c}	260^{c}	26.7	<0.001	0.213	0.039	<0.001
White clover (g/kg DM)	164	145	157	144	17.1	0.813	0.776	0.009	0.107
Weeds (g/kg DM)	11	13	8	11	3.1	0.733	0.598	0.890	0.195
Dead materials (g/kg DM)	160^{a}	137^{b}	106^{c}	106^{c}	4.9	<0.001	0.117	0.513	0.793
DM (g/kg FW)	270^{a}	228^{b}	208^{c}	204^{c}	3.3	<0.001	<0.001	0.590	0.022
CP (g/kg DM)	183	186	189	189	2.8	0.361	0.920	0.220	0.827
CF (g/kg DM)	28^{a}	27^{b}	27^{b}	26^{b}	0.4	<0.001	0.258	0.483	0.706
ADF (g/kg DM)	269^{a}	261^{b}	254^{c}	251^{c}	2.7	<0.001	0.760	0.545	0.521
NDF (g/kg DM)	469^{a}	443^{b}	408^{c}	407^{c}	5.9	<0.001	0.983	0.384	0.639
NSC (g/kg DM)	210^{a}	232^{b}	259^{c}	260^{c}	4.6	<0.001	0.897	0.502	0.601
OMD (g/kg DM)	704^{a}	722^{b}	731^{b}	730^{b}	6.0	0.006	0.498	0.760	0.991
ME (MJ/kg DM)	10.1	10.2	10.4	10.4	0.07	0.075	0.384	0.814	0.696
Ca (g/kg DM)	8.3^{a}	11.7^{b}	14.3^{c}	14.4^{c}	0.47	<0.001	0.270	0.264	0.145
K (g/kg DM)	23.5	23.9	24.1	24.9	0.61	0.418	0.515	0.013	0.842
Na (g/kg DM)	3.5^{a}	5.0^{b}	5.8^{b}	5.7^{b}	0.29	<0.001	0.033	0.102	0.018
Cl (g/kg DM)	12.3^{a}	15.0^{b}	16.3^{b}	16.3^{b}	0.62	<0.001	0.352	0.853	0.335
Aucubin (g/kg DM)	0.62^{a}	3.36^{b}	5.20^{c}	5.93^{c}	0.25	<0.001	0.002	<0.001	<0.001
Acteoside (g/kg DM)	0.6^{a}	5.2^{b}	9.1^{c}	10.2^{c}	0.6	<0.001	0.004	<0.001	<0.001

Note: $^{a, b, c, d}$ Means within a row with different superscripts differ at $P < 0.05$, ADF = acid detergent fibre, CF = crude fat, CP = crude protein, DMI = DM intake, FW = fresh weight, ME = metabolisable energy, NDF = neutral detergent fibre, NSC = non-structural carbohydrates, OMD = organic matter digestibility, RGWC = perennial ryegrass and white clover, PLL = RGWC + low plantain rate, PLM = RGWC + medium plantain rate, PLH = RGWC + high plantain rate, S = season, SEM = standard error of the means, T = treatment, Y = lactation year.

5.3.2. Milk yield and milk composition

The effect of pasture treatment and its interactions with season and year on the intake and milk variables over the experimental period are presented in Table 5.3 and Appendix 5. Overall, the treatment did not affect DMI intake, milk yield, the composition and yield of milk solids, milk protein, milk fat, or milk lactose (P > 0.05); however, it significantly influenced the content of MUN (P < 0.05). On average, cows had 3.7, 5.7 and 6.4 kg DM plantain per day in PLL, PLM and PLH, accounting for 17, 25 and 28% of the apparent diet, respectively. Over two lactation years, cows grazing RGWC, PLL, PLM, and PLH had an average of 25.7 L daily milk yield, 3.36 kg milk solids, 0.85 kg milk protein, 1.02 kg milk fat, and 1.35 kg milk lactose. On average, the MUN of cows grazing the plantain-based pastures was lower than that in RGWC by 10–17% (P <0.05).

Table 5.3. Milk yield and milk compositions of dairy cows grazing pasture treatments over the experimental period.

	RGWC	**PLL**	**PLM**	**PLH**	**SEM**	**P_T**	**$P_{T×S}$**	**$P_{T×Y}$**	**$P_{T×S×Y}$**
Herbage DMI (kg DM/cow/day)	15.5	16.0	16.4	17.1	0.49	0.129	0.704	0.780	0.930
Total apparent intake (kg DM/cow/day)	21.3	21.7	22.2	22.8	0.50	0.135	0.760	0.785	0.945
Dietary herbage content (%)	72.8	73.1	73.7	74.5	0.60	0.176	0.609	0.909	0.940
Herabge N intake (g N/cow/day)	456	456	478	497	15.2	0.164	0.325	0.560	0.676
Plantain intake (kg DM/cow/day)	0.53	3.74	5.71	6.40	0.24	<0.001	0.108	0.448	0.004
Plantain in the diet (%)	2.4	17.0	25.3	27.8	0.96	<0.001	0.103	0.356	0.001
Milk yield (L/cow/day)	25.5	25.6	25.7	25.9	0.25	0.698	0.396	0.882	0.337
Milk solid (%)	13.7	13.8	13.7	13.8	0.09	0.623	0.058	0.141	0.009
Milk solids (kg/cow/day)	3.33	3.35	3.36	3.40	0.04	0.665	0.221	0.245	0.076
Protein (%)	3.43	3.47	3.41	3.46	0.02	0.081	0.387	0.575	0.196
Protein (kg/cow/day)	0.84	0.85	0.84	0.86	0.01	0.310	0.045	0.950	0.479
Fat (%)	4.20	4.17	4.17	4.25	0.09	0.930	0.555	n.a	n.a
Fat (kg/cow/day)	1.03	1.01	1.02	1.04	0.02	0.881	0.053	n.a	n.a
Lactose (%)	4.98	4.98	4.96	4.97	0.02	0.788	0.779	n.a	n.a
Lactose (kg/cow/day)	1.34	1.35	1.35	1.36	0.02	0.914	0.440	n.a	n.a
MUN (mg/dL)	10.2^a	9.2^b	8.5^c	8.7^c	0.10	<0.001	0.001	0.001	0.001

Note: $^{a, b, c, d}$ means within a row with different superscripts differ at P < 0.05, MUN = milk urea nitrogen, n.a = variables were measured only in one year (2020/2021), RGWC = perennial ryegrass and white clover, PLL = RGWC + low plantain rate, PLM = RGWC + medium plantain rate, PLH = RGWC + high plantain rate, S = season, SEM = standard error of the means, T = treatment, Y = year.

5.3.3. Nitrogen excretion

The characteristics of urine and dung of dairy cows grazing different pastures over the study period are shown in Table 5.4 and Appendix 5. Cows grazing the plantain-based pastures had a lower UN concentration by 15–27% and a lower urinary creatinine concentration by 17–27%, compared with cows grazing the RGWC pasture ($P < 0.05$). The lower urinary creatinine resulted in 22–40% higher urine volume from cows grazing the plantain-based pastures ($P < 0.05$). Furthermore, grazing the pastures containing plantain caused an increase of 4–9% in faecal N concentration ($P < 0.05$) and a decrease of 1–6% in faecal DM content ($P < 0.05$). In addition, sodium, potassium and chloride excretion in the urine of cows grazing the plantain-based pastures were higher than those grazing RGWC ($P < 0.05$). Among treatments, all plantain rates (PLL, PLM, PLH) significantly affected UN concentration, urinary creatinine, urine volume, sodium, potassium and chloride excretion in the urine, and faecal N concentration. At the same time, only the medium (PLM) and high (PLH) rates of plantain reduced faecal DM content ($P < 0.05$).

Regarding N partitioning, grazing the plantain-based pastures reduced N partitioned to urine ($P < 0.05$) and increased N partitioned to dung ($P < 0.05$), but it did not affect N partitioned to milk ($P > 0.05$). Specifically, cows grazing the plantain-based pastures had a 4–9% lower UN excretion and a 3–7% higher FN excretion than those grazing RGWC ($P < 0.05$). Among treatments, the medium (PLM) or high rate (PLH) of plantain decreased UN excretion ($P < 0.05$), and only the high rate of plantain (PLH) significantly increased faecal N excretion.

Table 5.4. Characteristics of urine and faeces, and nitrogen (N) excretion of dairy cows grazing pasture treatments over the experimental period.

	RGWC	PLL	PLM	PLH	SEM	P_{T}	$P_{T×S}$	$P_{T×Y}$	$P_{T×S×Y}$
Urine									
N (%)	0.62^{a}	0.53^{b}	0.46^{c}	0.45^{c}	0.02	<0.001	0.024	0.680	0.075
Urea (mmol/L)	220^{a}	193^{b}	170^{c}	178^{c}	4.8	<0.001	<0.001	0.246	0.238
Creatinine (mmol/L)	4.01^{a}	3.31^{b}	3.00^{c}	2.93^{c}	0.09	<0.001	0.207	0.757	0.240
Na (g/day)	54.5^{a}	70.4^{b}	83.1^{b}	78.3^{ab}	3.0	<0.001	0.002	0.483	0.004
K (g/day)	203^{a}	234^{b}	255^{c}	270^{d}	5.2	<0.001	0.021	0.010	<0.001
Cl (g/day)	133^{a}	201^{b}	212^{bc}	225^{c}	5.8	<0.001	<0.001	0.005	<0.001
Faeces									
DM content (%)	12.0^{a}	12.1^{ab}	12.5^{bc}	12.7^{c}	0.22	0.053	0.289	0.639	0.088
N (%)	2.43^{a}	2.53^{b}	2.55^{b}	2.64^{c}	0.02	<0.001	0.872	0.561	0.714
FNP (%)	0.19^{a}	0.21^{b}	0.20^{b}	0.20^{b}	0.003	0.001	0.196	0.300	0.032
N excretion									
Urine volume (L/day)	28.7^{a}	35.1^{b}	39.3^{c}	40.3^{c}	1.4	<0.001	0.073	0.011	0.513
Urine N (g/day)	180^{a}	174^{ab}	164^{b}	167^{b}	3.6	0.004	0.012	0.029	0.373
Milk N (g/day)	116	119	121	119	1.7	0.094	0.009	0.862	0.130
Faecal N (g/day)[1]	121^{a}	124^{a}	124^{a}	130^{b}	1.4	<0.001	0.317	0.842	<0.001

Note: [a, b, c, d] Means within a row with different superscripts differ at P < 0.05, SEM = standard error of the means, T = treatment, S = season, Y = year, [1] FN excretion was estimated using the total DMI, including herbage and supplement intake, assuming cows consumed 90% of offered supplements, RGWC = perennial ryegrass and white clover, PLL = RGWC + low plantain rate, PLM = RGWC + medium plantain rate, PLH = RGWC + high plantain rate.

5.3.4. Effect of lactation season

The interactions between treatment and lactation season significantly affected UN concentration, urine volume (tendency, P < 0.1), UN excretion and MUN (P < 0.05, Table 5.4 and Figure 5.1). The effect of pasture treatment on UN concentration, urine volume, N partitioned to urine, and MUN in late summer and autumn was greater than in early summer (P < 0.05). For instance, grazing the plantain-based pastures (on average of PLL, PLM and PLH) resulted in a reduction in UN concentration by 27 and 28%, a decrease in UN excretion by 8 and 10%, and an increase in urine volume by 37 and 35% in late summer and autumn, respectively. In early summer, cows with pastures containing plantain (on average of PLL, PLM and PLH) had a lower UN concentration by 17% ($P < 0.05$), a similar UN excretion ($P > 0.05$), and an increase in urine volume by 28%, compared with the RGWC pasture ($P < 0.05$). The effect of grazing the plantain-based pastures on MUN in early summer was smaller than in late summer and autumn ($P < 0.05$). Compared with grazing RGWC, cows grazing the plantain-based pastures (on average of PLL, PLM and PLH) had a lower MUN by 8% in early summer, 14% in late summer and 17% in autumn.

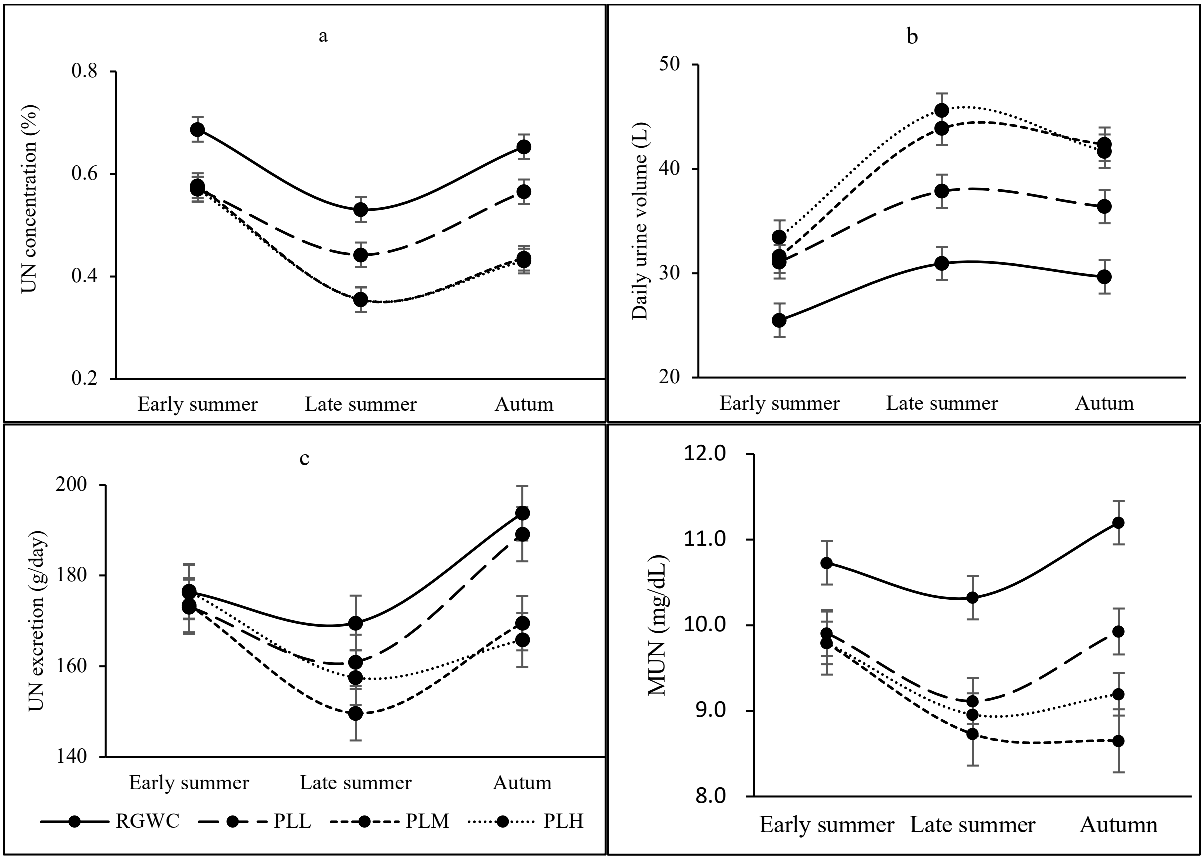

Figure 5.1. Urine nitrogen (UN) concentration (a), daily urine volume (a), UN excretion (c), and milk urea N (MUN) (d) of dairy cows grazing pasture treatments in different seasons over the experimental period.

Note: RGWC = perennial ryegrass-white clover, PLL = RGWC + low plantain rate, PLM = RGWC + medium plantain rate, PLH = RGWC + high plantain rate.

5.3.5. The effect of plantain content

The relationship between plantain content and the relative changes in UN concentration, urine volume, UN excretion and FN excretion are shown in Table 5.5 and Figure 5.2. In general, dietary plantain content was negatively associated with the relative change in UN concentration ($P < 0.05$, $R = -0.82$), positively correlated with the relative change in urine volume ($P < 0.05$, $R = 0.84$), and did not significantly affect the UN and FN excretion ($P > 0.05$). The percentage of plantain leaves in the diet explained 70% of the change in urine volume (linear regression model: RC in urine volume (%) = 1.19 × plantain in the diet (%) + 1.69), and 67% of the change in UN concentration (linear regression model: RC in urine volume (%) = -0.88 × plantain in the diet (%) - 1.57) (Figure 5.2). In addition, a strong relationship was observed between the relative change in urine volume and the relative change in UN concentration (negative, R = -0.96), the relative change in urine volume and the relative change in UN output (negative, R = -0.48), the relative change in UN concentration and the relative change in UN output (positive, R = 0.64).

Table 5.5. Pearson correlation between the content of plantain leaves (%) and the relative change in urinary nitrogen (UN) concentration, urine volume, UN excretion, and faecal N excretion.

		Plantain leaves (%)	Relative change in UN concentration	Relative change in Urine volume	Relative change in UN excretion
Relative change in UN concentration	R	- 0.82			
	P-value	<0.001			
Relative change in urine volume	R	0.84	- 0.96		
	P-value	<0.001	<0.001		
Relative change in UN excretion	R	- 0.25	0.64	- 0.48	
	P-value	0.231	0.001	0.018	
Relative change in FN excretion	R	0.39	- 0.34	0.34	- 0.14
	P-value	0.063	0.103	0.106	0.500

Note: R = Pearson correlation coefficient; relative change = 1 - Y_{PLi}/Y_{RGWC}, where Y_{PLi} and Y_{RGWC} refer to the mean value of cows grazing treatment i and RGWC in a grazing, respectively.

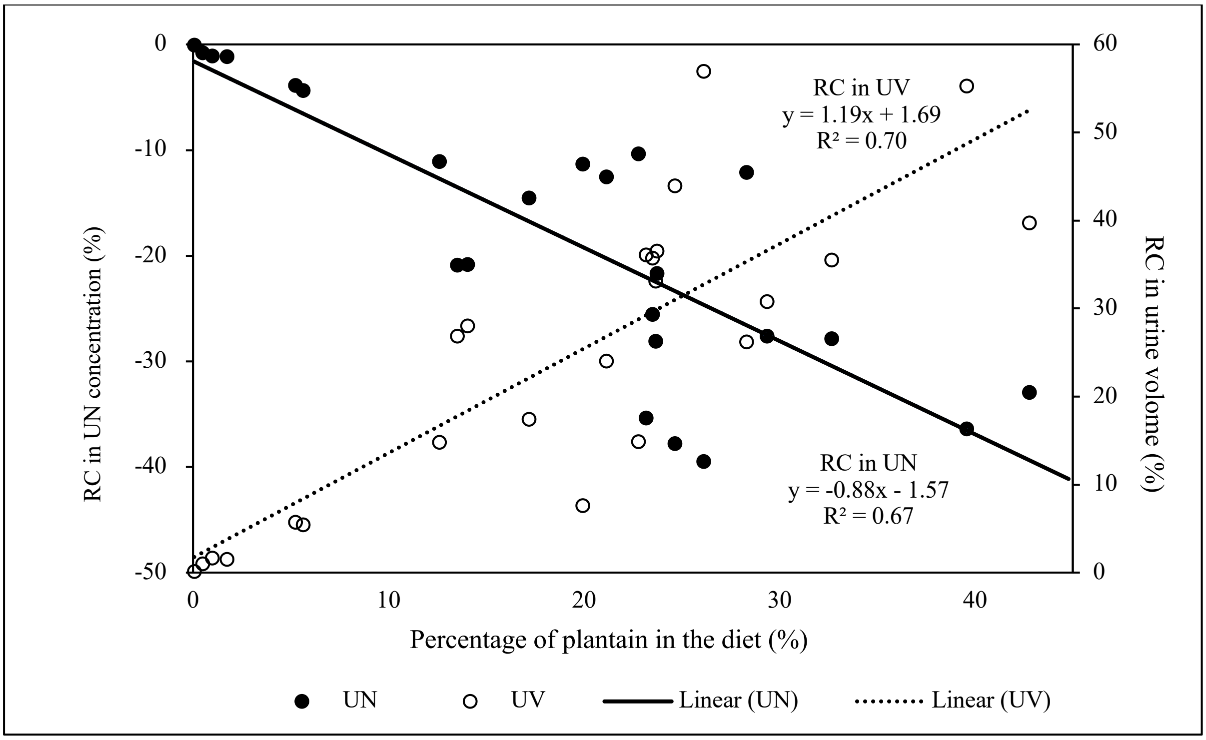

Figure 5.2. Relationship between the content of plantain leaves in the diet (%) and the relative change (RC) in urinary nitrogen (UN) concentration and urine volume over the experimental period.

5.4. Discussion

Previous studies have found that including plantain in the diets of dairy cows reduces N concentration in the urine and increases the urine volume while increasing the milk yield of dairy cows under research conditions (McCarthy et al., 2020; Nguyen et al., 2022a). However, the effect of incorporating different proportions of plantain into pastures on milk production and UN excretion in a typical farm condition over an extended period will provide farmers with greater confidence to integrate plantain with traditional pastures to improve animal performance and environmental benefits. The present study suggests that under a 'typical' practice offering mainly grazed pastures, and up to 25% concentrate feeds, incorporating plantain with RGWC reduced the risk of N leaching from pastoral systems and maintained the milk production of dairy cows.

5.4.1. Reducing the risk of N leaching

The results of the present study support the hypothesis that incorporating plantain into RGWC pasture can reduce the risk of N losses from pastoral systems (MPI, 2021; Navarrete S., 2018). Firstly, grazing plantain-based pasture could reduce the amount of UN deposited onto soils by grazing cows, decreasing the risk of NO_3^- leaching and nitrous oxide emissions from the systems (Carlton et al., 2019; Marsden et al., 2016). Secondly, incorporating plantain can increase urine production of cows, spreading UN over larger urine patches with a lower UN load, allowing pastures to take up more N from pastoral soils (Cameron et al., 2013; Romera et al., 2012). In addition, plantain may shift more dietary N to stable forms in dung rather than vulnerable forms in urine, meaning that more N can be converted to organic N in soils and used by pastures, and lesser N losses into the environment (Petersen et al., 1998). The clear effect of plantain on reducing N concentration and increasing urine production suggests that the dilution of UN is an important factor for reducing N losses from grazed paddocks. APSIM model indicated that the decrease of 40% in UN concentration under a similar UN excretion caused a reduction in NO_3^- leaching by 50% from urine patches and 22% from grazing paddocks (Ledgard et al., 2015). The combination of reducing UN concentration by 15–27%, increasing urine volume, and reducing UN excretion suggests that incorporating 24–38% plantain into the RGWC pasture can reduce NO_3^- leaching by a significant amount. A direct measurement of NO_3^- leaching and nitrous oxide emission at paddock scale in associated with the change in N depositing by animals is required to quantify the actual effect of plantain on N losses from pastoral systems.

The effect of plantain-based pastures on reducing UN concentration and UN excretion and increasing urine volume in the present study agrees with the previous studies conducted under research conditions and farm practice (Minnée et al., 2020; Navarrete et al., 2022). The meta-analysis using the data of short-term research in Appendix 1 or by Nguyen et al. (2022a) reported that a diet including an average of 43% plantain reduced UN concentration by 30% and UN excretion by 22%, while increased urine volume by 17%, with an influence of plantain content in the diet. At the same time, the measurement using urine sensors over two grazing events in Chapter 4 or by Nguyen et al. (2023) indicated that the diet containing 25% plantain decreased UN concentration by 29% and increased urine volume by 44%. Compared to the result from Chapter 4, the present study had a higher plantain content and lower effect on urine volume and UN concentration. There are two main reasons to explain this difference. The estimation of urine volume using the creatinine method may have underestimated the effect of plantain, possibly due to the difference in minerals and water intakes of cows across treatment diets, the frequency of urine sampling (Lee et al., 2019), or the varied effects of diets under different weather conditions (Khelil-Arfa et al., 2012; Romera et al., 2012; Shepherd et al., 2017). The effect of plantain incorporation on increasing urine production is often higher in late summer and autumn than in spring and early summer because the differences in DM content between plantain and RGWC pastures are more significant in drier seasons (as in Chapter 3 and by Nguyen et al. (2022b)). The results of the present research also align with previous studies that the decreased UN output of dairy cows when plantain is included in the cow diet was associated with an increased faecal N output (Carmona-Flores et al., 2020; Marshall et al., 2021; Minnée et al., 2020).

Nitrogen output in urine, faeces and milk in the present study was estimated using the data from spot samples and existing equations. The extensive dataset from various grazing over two lactation years allowed the present study to reliably report the effect of plantain on urine volume, UN concentration and UN excretion. However, spot sampling method cannot measure dinural changes that has been reported to influence plantain effect (Minnée et al., 2020). This can explain for a significant amount of unaccounted N from dietary N in the present study.

Therefore, comprehensive calibrations of creatinine equations and diurnal effect using urine sensors is important to improve the quantification of urine production and urination patterns by using spot samples.

The mechanisms for plantain to affect urine production and N partitioning by dairy cows have been intensively discussed in previous studies. The presence of bioactive compounds, aucubin and acteoside, and the high NSC content in plantain have been suggested to influence animal N partitioning. Specifically, aucubin and acteoside can inhibit ammonia production in cow rumen (Navarrete et al., 2016). Also, the high NSC content could improve the rumen synchrony of energy and protein for a higher milk yield and reduce protein degradability for a greater FN excretion (Bryant, Snow, et al., 2019; Totty et al., 2013). The present study found lower UN excretion and greater FN excretion but did not show a significant increase in N partitioned to milk. The increase in urine volume is likely due to the higher water content in plantain compared with RGWC (Bannink et al., 1999; Brown et al., 2022; Mangwe et al., 2019). Using the DM content and DMI, it can be estimated that cows grazing the plantain-based pastures had a higher herbage water intake by 11–23L than RGWC, nearly double the increase of 7–12 L urine volume in the present study. The limited resources did not allow the current study to measure water intake from drinking troughs and water excretion in dung to have a complete water balance. However, the high water intake from the herbage with plantain has been reported to reduce drinking water and faecal water output (Mangwe et al., 2019, Minnée et al., 2020), which can explain the lower difference in urine output compared with herbage water intake between treatments.

5.4.2. Maintaining milk production and composition

The inclusion of plantain in pastures has been shown to increase or maintain milk yield and milk composition in previous studies, depending on the difference in DMI and herbage quality between pastures and on the research conditions (Nguyen et al., 2022a). The results of the present study indicate that under a typical farm management practice offering 5–8 kg supplement per cow, incorporating an average of between 32–48% plantain with RGWC in the sward maintained milk yield, the concentration and milk of milk solids, protein, fat, and lactose over the two lactation seasons. These results agree with previous studies conducted under gazing conditions reporting no significant difference in DMI and energy intake (Navarrete et al., 2022; Nkomboni et al., 2021). In contrast, short-term studies conducted indoors and without providing supplements have reported that plantain increased milk solids production but reduced milk fat concentration (Box et al., 2017; Minneé et al., 2017). The increase in milk production has been shown to be associated with the higher apparent DMI and the greater NSC in plantain compared with RGWC. High NSC concentraton can provide rapid ready energy for microbial N production in the rumen and improve energy and protein balance to increase milk production (Bryant, Snow, et al., 2019; Totty et al., 2013). The lower NDF content in plantain may result in an insufficient supply of acetate for de novo fat production (Palmquist et al., 1993). Under the farm condition, like in the present study, the DMI was similar between treatments. Although the plantain-based pastures contained lower NDF and higher NSC concentration than RGWC, and the supplements such as maize silage and corn gluten pellets would have lowered NDF concentration in the total diet. As a result, the difference in NSC and NDF concentration from the pasture including 32-48% plantain over two years may be adequate to maintain milk yield and milk composition.

5.4.3. Effect of plantain content and season

The present study confirmed the hypothesis that a certain dietary plantain content is required to significantly affect UN excretion and urination dynamics. Also, these effects are associated with the percentage of plantain in the diet (Minnée et al., 2020; Nguyen et al., 2022a). Previous short-term studies have agreed that the diet needs to include at least 30% plantain to reduce UN concentration (Bryant et al., 2018; Minnée et al., 2020; Nkomboni et al., 2021) and ≥40% plantain to increase urine volume and UN excretion (Box et al., 2017; Minnée et al., 2020; Minneé et al., 2017). However, the measurement using urine sensors in Chapter 4 reported a significant effect of 18% plantain on decreasing UN concentration and 25% plantain on increasing UN volume of dairy cows, managed under a similar condition to the present study (Nguyen et al., 2023). The effective proportion of dietary plantain in the present study (17% to reduce UN concentration and 28% to decrease UN excretion and increase urine volume) is almost similar to 18–25% in Chapter 4 and Nguyen et al. (2023) but lower than in those short-term studies. In single measurements with small sample size, a larger proportion of plantain is often required to cause a statistical difference in urine volume, UN concentration and UN excretion between experimental and control animals because these variables are highly varied between cows and sampling times (Betteridge et al., 2013; Tebbe & Weiss, 2018). The intensive measurement with repeated grazing events and replicated cows may have enabled the present study to find significant effects with a lower percentage of plantain in the diet. Regression models can estimate the changes in urine volume, UN concentration and UN excretion when plantain content is identified. The results of the present study suggest that using plantain content can provide a reliable estimation of the relative changes in urine volume and UN excretion from lactating cows. However, the effect of plantain on UN and FN excretion was too small to be estimated accurately by the proportion of plantain in the diet. In grazing dairy systems, it will become important for farmers to maintain more than 30% plantain in the pasture over an extended period (Dodd et al., 2019; Nguyen et al., 2022b). Together with the results of Chapter 3 and Nguyen et al. (2022b), the present study suggests that incorporating medium (PLM) or high (PLH) plantain rates is better than low plantain (PLL) rate in reducing the risk of N losses from pastoral systems for at least two years following establishment.

Regarding seasonal effect, the results of the present study agree with Navarrete et al. (2022) and Box et al. (2017) the impact of plantain inclusion on UN concentration, urine volume and UN excretion is greater in late lactation than in early lactation. The main drivers for this variation could relate to the change in the composition and quality of plantain and ryegrass over seasons. According to Nguyen et al. (2022b) and the results of Chapter 3, the difference in DM content, bioactive compounds, and carbohydrate profiles between plantain-based pasture and RGWC pasture in summer and autumn is greater than in spring and early summer. Pastures containing the same proportion of plantain may result in greater herbage water intake of grazing animals, therefore increasing urine volume in late lactation (Bannink et al., 1999; Khelil-Arfa et al., 2012). The greater intake of aucubin, acteoside and NSC from plantain-based pasture than RGWC pasture in late summer and autumn can also cause a greater reduction in UN excretion during these periods (Black, 1971; Navarrete et al., 2016). In addition, the high proportion of plantain reproductive stem and seed heads in early summer may reduce the intake of plantain because cows prefer to select palatable ryegrass and plantain leaves rather than the lower-quality plantain stem and seed heads (Navarrete, 2015). The greater effect of plantain-based pasture on reducing UN excretion and urine production in late lactation can expand the reduction in N losses from pastoral systems for the whole year. This is because late summer and autumn are the critical periods determining N losses in grazed systems (Christensen et al., 2018). This result indicates the importance of maintaining at least 20% plantain in summer and autumn to enhance the benefit of plantain incorporation.

5.5. Conclusions

Under a typical dairy farm practice offering mainly grazed pastures and up to 25% supplements, incorporating an average of 32% plantain with RGWC reduced UN excretion by 7%, decreased UN concentration by 22%, increased urine volume by 33%, and did not affect milk yield and composition. The changes in UN concentration and urine volume were strongly linear with the percentage of plantain in the diet. In addition, the effect of the plantain-based pastures on UN concentration, urine volume and UN excretion in late summer and autumn was more significant than in early summer. The results of the present study suggest that incorporating plantain with RGWC potentially reduces the risk of N leaching from pastoral systems while maintaining the milk production of dairy cows.

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CHAPTER 6

IMPACT OF PLANTAIN-BASED PASTURE ON NITRATE LEACHING FROM PASTORAL SYSTEMS

Chapters 4 and 5 demonstrated that plantain-based pasture increased urine production and urinary frequency while reducing urine nitrogen excretion of dairy cows, potentially affecting nitrate (NO_3^-) leaching from pastoral systems. Chapter 6 compares NO_3^- leaching from the pastoral systems with and without plantain.

A paper from this chapter is in preparation to submit to Science of The Total Environment as:

Nguyen, T.T.; Navarrete, S.; Horne, D.; Donaghy, D.; Kemp, P. Incorporation of plantain with perennial ryegrass-white clover reduces nitrate leaching from a pastoral dairy system.

Highlights

- Incorporating 30 and 50% plantain resulted in a 32 and 52% decrease in NO_3^- leaching.
- Nitrate leaching was decreased with increasing plantain contents in the sward.
- Reduced NO_3^- leaching was linked with increasing N uptake and decreasing UN load.
- Managing plantain content is necessary for the long-term mitigation of NO_3^- leaching.

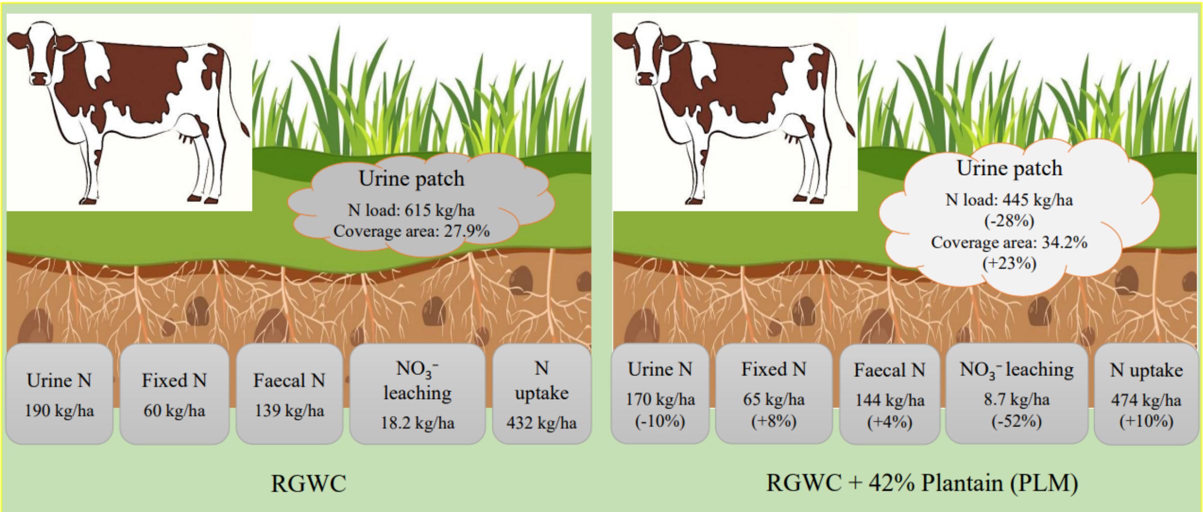
Abstract

There is increasing interest in incorporating plantain into perennial ryegrass-white clover (RGWC) pasture to overcome feed deficit during late summer/autumn and reduce nitrate (NO_3^-) leaching from pastoral dairy systems. However, the impact of plantain-based pastures on nitrogen (N) losses at the farm scale is not fully understood. An experiment was conducted to quantify the effect of increasing the proportion of plantain in RGWC swards on NO_3^- leaching. Pasture treatments were RGWC, RGWC + low plantain rate (PLL), RGWC + medium plantain rate (PLM), and RGWC + high plantain rate (PLH). Five replicates of each treatment were established on 20 experimental plots and four adaptation paddocks. The soil at the experimental site is Pallic soil, and each plot has an independent mole-pipe drainage system. Pastures were grazed by dairy cows over 22 grazing events between September 2019 and December 2023. At each grazing, 60 or 80 cows were assigned to four groups, adapted to their treatment diets over 6 days in adaptation paddocks before grazing the plots for 1.5–3.0 days. Drainage quantity and quality were measured at the outlet of the drainage system of each plot. Herbage N uptake, biological N fixation, urine and faecal N excretion, urine patch area, and N load to urine patches were estimated from pasture, urine, and faecal samples. The results showed that incorporating an average of 30 and 50% plantain with RGWC decreased NO_3^- leaching by 32 and 52% relavent to RGWC, respectively, over the two years, with a greater reduction in the first year than in the second year. Across treatments, PLM had the lowest NO_3^-

leaching with a decrease of 64% in the first year and 41% in the second year, compared to RGWC, respectively. The reduction in NO_3^- leaching was associated with a larger urine patch area, a lower N load in urine patches and a higher herbage N uptake. The results of the present study suggest that incorporating plantain into the RGWC pasture can be an effective strategy to mitigate NO_3^- leaching from pastoral dairy systems for at least two years from establishment.

Graphical abstract

Plantain-based pasture reduces NO_3^- leaching from a pastoral dairy system



Keywords: Herbage N uptake, nitrogen losses, plantain-based pasture, plantain content, *Plantago lanceolata,* urine patch.

6.1. Introduction

Nitrate (NO_3^-) leaching from pastoral dairy systems adversely impacts water quality worldwide (Di & Cameron, 2002). The primary source of leached NO_3^- in grazing-based dairy production systems is nitrogen (N) in animal urine deposited as localised patches onto pastoral soils (Selbie et al., 2015). In grazed systems with perennial ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) (RGWC) pasture, the average N loading rate at urine patches of dairy cows is approximately 610 kg/ha (Selbie et al., 2015). This high N load exceeds the uptake capacity of the pasture; consequently, the surplus N is at risk of being leached during drainage events (Cameron et al., 2013). Regulations have been developed to limit the amount of N lost from dairy farms, increasing the interest in mitigation strategies for NO_3^- leaching (Minister for Housing Planning Local Government and Heritage, 2022; Minister for the Environment, 2020).

In recent years, plantain (*Plantago lanceolata*) has been used in grazed pastures to reduce N losses from dairy systems without adversely affecting farm productivity. Lysimeter studies have indicated that incorporating plantain into traditional pastures decreases NO_3^- leaching by 74–89% from urine patches (Carlton et al., 2019; Woods et al., 2018). In comparison, at the farm scale, NO_3^- leaching from pure plantain or pastures containing plantain can be 48–56% less than the losses under RGWC pasture (Al-Marashdeh et al., 2021; Rodriguez et al., 2020). The reduction in NO_3^- leaching is influenced by the content of plantain and clover in the sward (Rodriguez, 2020). There are several mechanisms by which plantain decreases N leaching (Bryant, Snow, et al., 2019). For example, the high water content in plantain can increase urine volume and the number of urine patches, thereby spreading UN across larger areas (Mangwe et al., 2019; Nguyen et al., 2022a); consequently, pastures can uptake a greater proportion of the N from urine patches (Woods et al., 2018). In addition, bioactive compounds such as aucubin and acteoside in plantain impact nitrification processes, inhibiting NO_3^- production in the soil (Gardiner et al., 2017; Rodriguez et al., 2021). Although plantain is used in combination with RGWC pasture on dairy farms, there is a lack of quantitative knowledge about the effect of this mixed sward on NO_3^- leaching at the farm scale and in particular the relationship between the proportion of plantain in the sward and the reduction in the leaching.

The aim of the present study was to quantify the effect of increasing the proportion of plantain in a mixed RGWC pasture on NO_3^- leaching from a pastoral dairy system, to provide an account of this leaching in terms of the impact of plantain on the N cycle, and to identify the required proportion of plantain in the sward to reduce NO_3^- leaching.

6.2. Materials and Methods

6.2.1. Experimental site, treatment and management

The study was conducted at Massey University's No. 4 Dairy Farm in Palmerston North, New Zealand (40°23'26.9"S, 175°36'43.5"E) from April 2019 to December 2021. Grazing management and animal samplings were implemented according to the procedure approved by Massey University Animal Ethics Committee (19/54). The study site is on Tokomaru silt loam soil, a Pallic soil (Hart et al., 1988). The climate at the site is temperate. The mean annual rainfall is 950 mm. The details of soil characteristics and pasture establishment are described in Chapter 3.

The experiment was a completely randomised design with four pasture treatments and five replicate plots. An isolated mole and pipe drainage system was set up in each plot before the pasture was established. Mole channels were ploughed at 2 m intervals at a 0.5 m depth. Moles were connected to a 0.11 m diameter pipe drain, installed in a 0.6 m deep trench and covered by gravel. Tipping buckets were set up at the outlet of the drainage system of each plot to monitor drainage. All drainage water from each plot flowed through the drainage system into the tipping buckets, and a counter recorded the number of drainage tips from each plot. A plastic container attached to the system collected approximately 0.1% drainage water from every second tip giving a flow-proportioned sample. The pasture treatments were perennial ryegrass (cv. ONE^{50})-white clover (cv. Tribute), RGWC + low plantain (cv. Agritonic) rate (PLL), RGWC + medium plantain rate (PLM), and RGWC + high plantain rate (PLH). The sowing rate of plantain, ryegrass and white clover was 0, 20 and 3 kg/ha for RGWC, 4, 15 and 3 kg/ha for PLL, 7, 10 and 3 kg/ha for PLM, and 10, 5, 3 kg/ha for PLH, respectively. The pasture was established on 5 April 2019 with four adaptation paddocks (1-ha paddock × 4 treatments) and 20 experimental plots (five 800 m^2 plots × 4 treatments). The details of the establishment and sowing rates were described by Nguyen et al. (2022b).

Table 6.1. Phases and dates of management and measurements over the experimental period.

Period	Date	Management	Measurements
Establishment	5 Apr	Sowing	
Apr-Dec 2019	28 Jun	Lax grazing	
	11–19 Sep	Grazing (4 cows × 2 days/plot)	DM, BC, PN
	8 Oct	Fertilising 50 kg N/ha	
	22 Oct	Mowing	
	16–24 Oct	Grazing (4 cows × 2 days/plot)	DM, BC
	13–21 Nov	Grazing (4 cows × 2 days/plot)	DM, BC
	4–12 Dec	Grazing (4 cows × 2 days/plot)	DM, BC, PN, AN
	20 Dec	Fertilising 50 kg N/ha	
Year 1	8–17 Jan	Grazing (4 cows × 3 days/plot)	DM, BC
Jan-Dec 2020	21 Jan	Mowing	
	5–12 Feb	Grazing (3 cows × 1.5 days/plot)	DM, BC, PN, AN
	21 Feb	Fertilising 50 kg N/ha	
	12–19 Mar	Grazing (3 cows × 2 days/plot)	DM, BC, PN, AN
	7–15 May	Grazing (3 cows × 3 days/plot)	DM, BC
	1–9 Jul	Grazing (3 cows × 3 days/plot)	DM, BC
	26 Aug–3 Sep	Grazing (3 cows × 3 days/plot)	DM, BC, PN
	17 Sep	Fertilising 30 kg N/ha	
	7–15 Oct	Grazing (4 cows × 3 days/plot)	DM, BC, PN
	16 Oct	Mowing	
	11–19 Nov	Grazing (4 cows × 3 days/plot)	DM, BC, PN
	20 Nov	Fertilising 30 kg N/ha	
	2–10 Dec	Grazing (3 cows × 2 days/plot)	DM, BC, PN, AN
	11 Dec	Mowing	
Year 2	6–15 Jan	Grazing (4 cows × 3 days/plot)	DM, BC
Jan-Dec 2021	3–11 Feb	Grazing (3 cows × 2 days/plot)	DM, BC, PN, AN
	23–31 Mar	Grazing (3 cows × 2 days/plot)	DM, BC, PN, AN
	7 Apr	Spray to remove ryegrass in PLH	
	21 Apr	Fertilising 30 kg N/ha, excepted PLH	
	22 Apr	Direct drilling of 3 kg ryegrass, 10 kg plantain, and 3 kg white clover in PLH	
	5–13 May	Grazing (3 cows × 3 days/plot), except PLH	DM, BC, PN, AN
	23–30 Jun	Grazing (3 cows × 3 days/plot), except PLH	DM, BC
	3–10 Sep	Grazing (3 cows × 2 days/plot)	DM, BC
	7–15 Oct	Grazing (4 cows × 2.5 days/plot)	DM, BC
	22 Oct	Fertilising 30 kg N/ha	
	4–12 Nov	Grazing (3 cows × 2.5 days/plot)	DM, BC
	12 Nov	Mowing	
	2–10 Dec	Grazing (3 cows × 2 days/plot)	DM, BC, PN, AN

Note: AN = urine and faecal nitrogen excretion, BC = botanical composition, DM = dry matter biomass, PLH = perennial ryegrass-white clover + high plantain rate, PN = nitrogen (N) concentration in pastures.

Pastures were grazed by dairy cows over 22 grazing events during the experimental period, including four grazings in the establishment period (2019) and nine grazings annually in 2020 (year 1) and 2021 (year 2). At each grazing, 60 or 80 cows (depending on the amount of pasture available) were assigned to graze each of the adaptation paddocks (15 or 20 cows/paddock) for 6 days before a subset of animals (3 or 4 cows/plot) grazed the corresponding plots for 1.5–3.0 days. Cows were allocated between 16-20 kg DM grazing pastures and between 5-8 kg DM supplements. The details of grazing management was described in Chapter 5.

By the beginning of the second year, the plantain content of PLH had declined to a similar level to that of PLM. Therefore, in April 2021, PLH was renovated to increase its plantain content. For this renovation, existing perennial ryegrass was removed by spraying with Sequence® (240g/L Clethodim) at 0.5 L/ha before the plots were resown with a seed rate of 3 kg perennial ryegrass, 10 kg plantain, and 3 kg white clover. To allow time for the new pastures to grow, this treatment was not grazed in May or June 2021. The period, key dates, management, and measurements during the experimental period are described in Table 6.1.

6.2.2. Climate data and soil water deficit simulation

The climate data for rainfall, temperature, wind speed, solar radiation and air humidity were obtained from a weather station (40°22’55.0"S, 175°36'32.9"E) 1 km from the study site. The soil water deficit was estimated using the soil water balance described by Scotter et al. (1979). In brief, the change in soil water storage was calculated by accounting for rainfall, and substracting evaporation, run-off and drainage water. The estimation of evaporation, run-off and drainage water was used data of field capacity in root zone, pasture coverage, temperature, wind speed, huminity, and solar radiation. The value of soil water deficit and drainage was zero (as soil is full capacity and no drainage, respectively) or negative.

6.2.3. Measurements of NO_3^- leaching and total N loss

Nitrate and total N leaching were calculated for individual plots via the drainage system. After a drainage event, the number of drainage tips from each plot was recorded. Drainage volume was calculated by multiplying the number of tips by the volume of each tip (5 L). Annual drainage volume (drainage depth) was cumulated from the volume of every drainage event between January and December. In addition, a drainage water sample (approx. 80 ml) was taken from the container back to the laboratory. Approximately 30 ml of the drainage sample was filtered through a 0.45 µm filter and analysed for NO_3^- concentration. Another subsample (approx. 10 ml) was digested to convert other N forms into NO_3^- in an autoclave at 120°C for an hour, using the persulphate digestion method (Hosomi & Sudo, 1986). The digested sample was analysed for total N concentration. The analysis of NO_3^- concentration was conducted on a Technicon Auto Analyser using the colourimetric method (Blakemore et al., 1981). The amount of NO_3^- and total N leaching in a drainage event from an individual plot was calculated by multiplying the drainage volume and N concentration in drainage water.

6.2.4. Measurement and estimation of herbage N uptake and biological N fixation

Herbage dry matter (DM) yield was measured for individual plots at every grazing by randomly cutting three samples (0.1 m^2 quadrat) to ground level before and after each grazing. The samples were oven-dried at 75 °C until a constant weight was achieved. The herbage yield was estimated by deducting the post-grazing DM mass at the previous grazing from the pre-grazing DM mass at the subsequent grazing. Botanical composition was measured for every grazing by taking a hand-plucked sample comprising 15–20 random grabs (approx. 400 g fresh weight) at approximately 5-7 cm heigt from each plot. Each sample was mixed thoroughly and subsampled into two smaller samples. The first subsample (approx. 100 g fresh weight) was manually separated into perennial ryegrass, white clover, plantain, weeds, and dead material. These components were oven-dried at 75 °C until a constant weight was achieved to calculate the composition of the pasture. The second subsample was oven-dried at 60 °C for 48 hours, ground through a 1 mm sieve, and analysed for herbage N content. The analysis of N content was conducted by a commercial laboratory using the near infra-red spectroscopy method (Hill Laboratory, Hamilton, New Zealand). Herbage N uptake and biological N fixation (BNF) for each year were estimated from the herbage yield and clover content between January and December using the following equations:

- Herbage N uptake (kg N/ha) = herbage yield (kg DM/ha) × herbage N content (%DM) (Sainju, 2017)
- BNF (kg/ha) = 28 × herbage yield (ton DM/ha) × clover content (%) (Lucas et al., 2010).

6.2.5. Measurements of urine and faecal N excretion, urine patch area and UN load

Urine N and faecal N (FN) excretions were measured for individual cows over seven grazing events in summer and autumn (February 2020, March 2020, December 2020, February 2021, March 2021, May 2021, and December 2021). Excreta were sampled in summer and autumn as most of the N leached from this soil is related to the quantity of N that accumulates under the urine patches which are deposited during these seasons (Christensen et al., 2018). Urine and faecal samples were collected from individual cows on two days when the cows were grazing the experimental plots, following the morning milking. Urine samples (approx. 80 ml each) were taken by vulva stimulation and subsampled into two smaller samples (approx. 30 ml each). The first subsample was acidified with sulfuric acid (6.0 normal) to reduce pH to below 4.0 and analysed for N concentration (Dumas method). The second subsample was analysed for creatinine content (Jaffe method). Faecal samples were taken by rectal stimulation and freeze-dried until a constant weight was achieved. The dried samples were ground to pass a 1 mm sieve and analysed for N concentration (Dumas method). All urine and faecal samples were stored at -20 ^{o}C until analysed. The analyses of these samples were conducted by a certified laboratory (Nutrition Laboratory, Massey University). Urine volume, the amount of N excretion in cow urine (UN) and dung (FN), urine patch area and UN load were estimated using the following equations:

- Urine volume (L/cow/day) = 21.9 × live weight (kg) ÷ urine creatinine (mg/kg).
- UN excretion (kg N/ha/year) = daily UN excretion (kg N/cow/day) × stocking rate (cow × day/ha/year),

  where daily UN excretion (g N/cow/day) = urine volume (L/cow/day) × UN content (g/L urine) (Pacheco et al., 2007).
- FN excretion (kg N/ha/year) = daily FN (kg/cow/day) × stocking rate (cow × day/ha/year),

  where daily FN (g/cow/day) = (DM intake (kg/day) × (1 - digestibility (%)) × FN content (g/kg dung) (Ahvenjärvi et al., 2018).
- Urine patch area (% plot) = (urine patch area - overlap area) ÷ plot area (Romera et al., 2012),

  where urine patch area (m^2/patch) = urine volume (m^3/cow/day) × stocking rate (cow × day/ha/year) ÷ urine column (m). A urine column of 10 mm was assumed (Selbie et al., 2015),

  and the overlap area was calculated using the probabilistic method and Poisson distribution, with the detailed calculation introduced in Romera et al. (2012).
- N load in urine patches (kg/ha) = total UN excretion (kg) ÷ total urine patch area (ha) (Romera et al., 2012).

6.2.6. Statistical Analysis

The experimental unit was individual plots for N loss and pasture variables (NO_3^- leaching, total N loss, drainage volume, NO_3^- and total N concentrations, botanical composition, herbage N uptake, and BNF) and was individual cows for animal variables (N excretion in urine and dung, urine patch area, and UN load). Data analysis for the N loss and pasture variables was conducted using the PROC mixed procedure of SAS 9.4 (SAS Institute, 2020) according to the model: $Y_{ijkl} = \mu + T_i + Y_j + (T \times Y)_{ij} + \beta I_k + P_l + e_{ijk}$, where: Y_{ijkl} = dependent variable; μ = overall mean; T_i = treatment i; Y_j = year j (January 2020 to December 2020 for year one, January 2021 to December 2021 for year two); $(T \times Y)_{ij}$ = the interaction between treatment i and year k; $\beta_1 I_k$ = covariate slope and baseline data of plot k in the establishment period (only for N loss variables); P_l = plot, the e_{ijkl} = residual error. Variables of N loss, such as drainage volume and drainage N concentration, are strongly affected by the slope and soil characteristics. Therefore, including the covariance with the established year data is vital to remove the effect of the plot. The analysis of animal variables (N excretion in urine and dung, urine patch area and UN load) was conducted using the model: $Y_{ijkl} = \mu + T_i + Y_j + (T \times Y)_{ij} + C_k + e_{ijk}$. Where, Y_{ijk} = dependent variable; μ = overall mean; T_i = treatment i; Y_j = year j; $(T \times Y)_{ij}$ = the interaction between treatment i and year k; C_l = cow, the e_{ijk} = residual error. A significant difference between means was declared at $P < 0.05$, and a tendency was reported at $P < 0.10$.

6.3. Results

6.3.1. Weather conditions

Annual rainfall in 2020 was 826 mm, notably lower than 1,084 mm in 2021. In 2020, the daily mean soil temperature at 10 cm depth was 13.9 ºC, ranging from 4.8 ºC to 24.4 ºC. In 2021, the daily mean soil temperature at 10 cm depth was 14.2 ºC, ranging from 4.5 ºC to 22.5 ºC.

Rains during winter and early spring meant that most of the drainage occurred between June and September. Little rain occurred during summer and autumn, resulting in a relatively large soil water deficit from February to May.

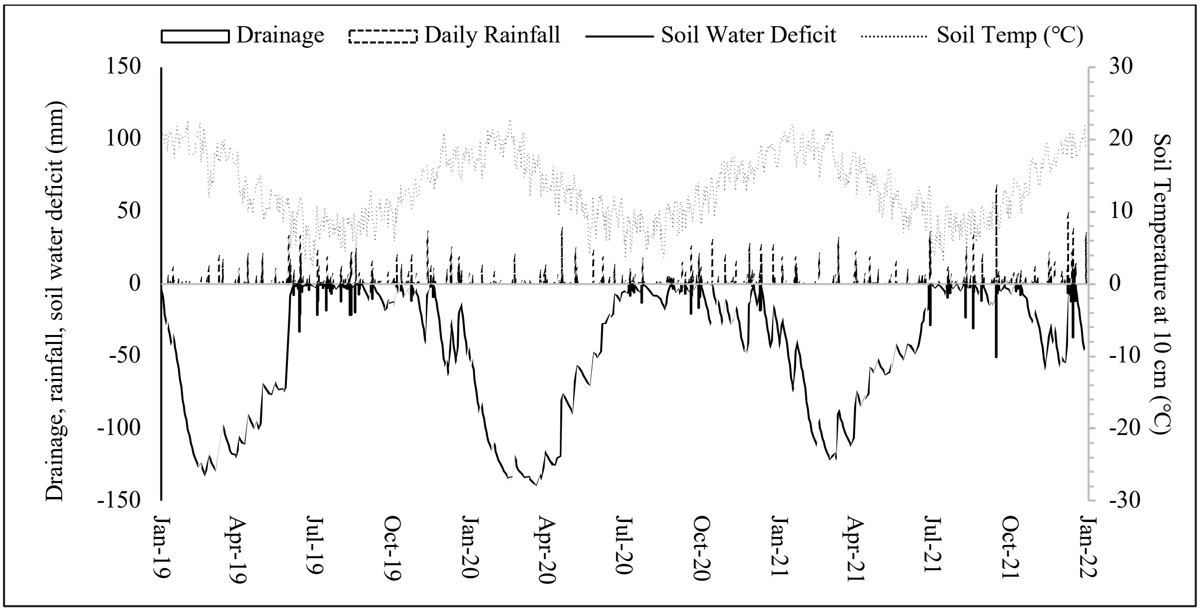


Figure 6.1. Daily rainfall, drainage, soil water deficit, and mean soil temperature at 10 cm between January 2019 and December 2021.

6.3.2. Nitrate and total N leaching

The NO_3^- and total N leaching significantly differed between treatments and years (Table 6.2, Figure 6.2 and Appendix 7). In 2020, the NO_3^- leaching under PLL, PLM, and PLH was lower by 55, 66 and 63%, respectively, than under RGWC. There was also a significant treatment effect on NO_3^- leaching in 2021, although decreases were lower, with 21, 46 and 32% less leaching in PLL, PLM and PLH, respectively, compared to RGWC. Over the two years, the amount of NO_3^- leached from PLL, PLM and PLH was 32, 52 and 42% less, respectively, than from RGWC ($P < 0.05$). Similarly, total N leaching from PLL, PLM and PLH was lower than from RGWC by 39, 32, and 36% in 2020, by 26, 36 and 28% in 2021, resulting in 29%, 35%, and 32% reduction over the experimental period ($P < 0.05$).

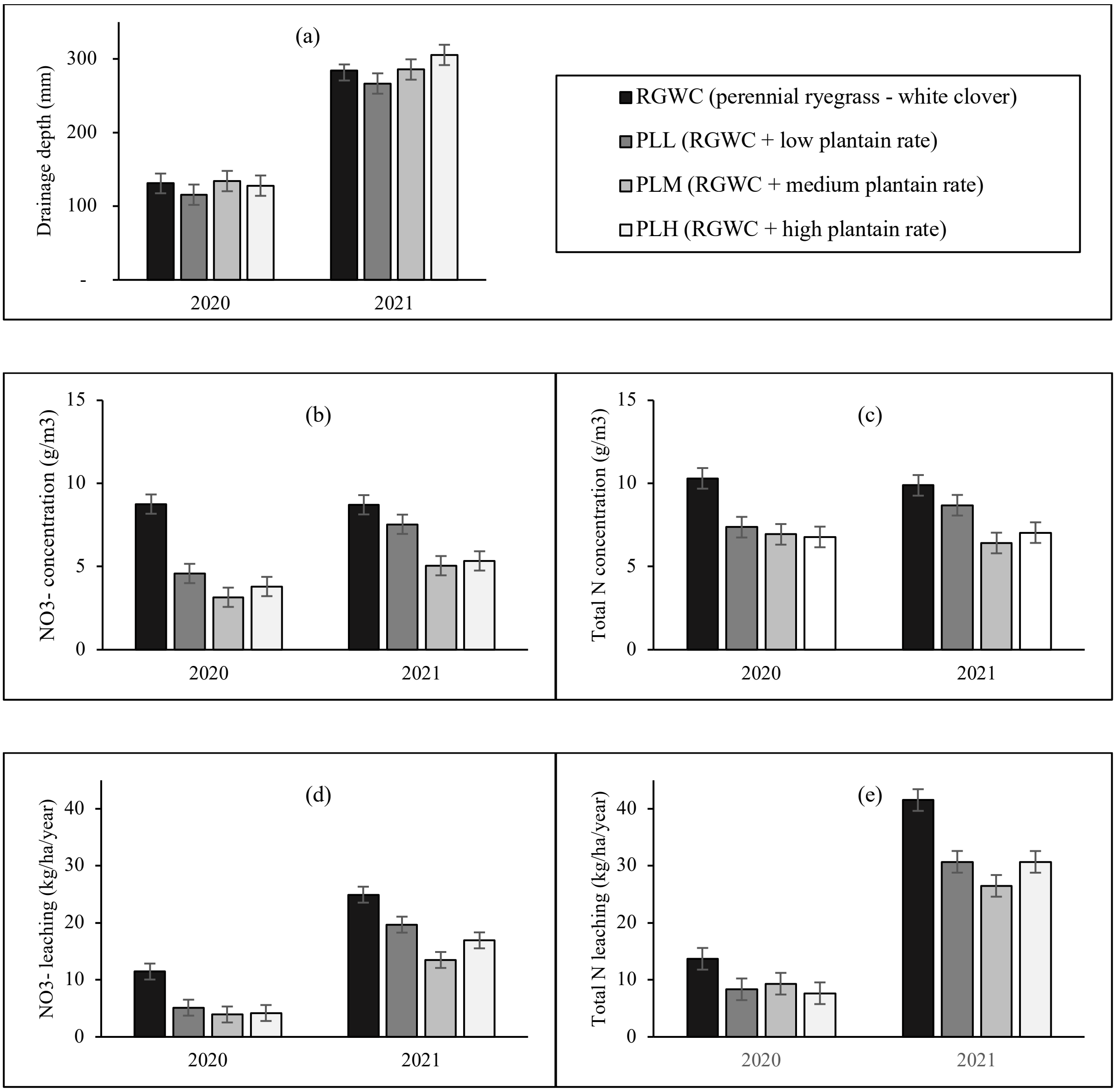


Figure 6.2. Annual drainage depth (a), nitrate (NO_3^-) concentration (b), total nitrogen (N) concentration (c), NO_3^- leaching (d), total N leaching (e) under pasture treatments in 2020 and 2021.

Annual drainage volume was similar among treatments over the experimental period and in each study year ($P > 0.05$), although 2020 was much drier than 2021. The average drainage volume across treatments in 2021 was 286 mm, more than double the value of 127 mm measured in 2020 (Figure 6.2, $P < 0.05$). The similar drainage volume across treatments meant that most of the recorded reduction in NO_3^- and total N leaching was due to lower NO_3^- and total N concentrations in drainage water. Averaged NO_3^- concentration in drainage from PLL, PLM and PLH treatments was lower than from RGWC by 47, 64, and 57% in 2020 ($P < 0.05$)

and by 14, 41 and 39% in 2021 ($P < 0.05$). Furthermore, the PLL, PLM and PLH treatments had a lower total N concentration in drainage water than the RGWC treatment by 28, 33, and 35% in 2020 ($P < 0.05$) and by 12, 35 and 30% in 2021 ($P < 0.05$). Over the experimental period, the mean NO_3^- concentration in PLL, PLM, and PLH was lower by 30, 53 and 47% than in RGWC ($P < 0.05$), while the mean total N concentration was lower in PLL, PLM and PLH by 21, 34 and 33% than in RGWC ($P < 0.05$), respectively.

The drainage pattern and the change in NO_3^- concentration across the drainage season notably differed between 2020 and 2021 (Figure 6.3). In 2020, drainage occurred between 13 July to 12 December. In 2021, the season started on 29 June and finished on 18 December. Cumulative drainage reached a value of 100 mm on 12 December in the first year and on 10 September in the second year. The NO_3^- concentration did not differ greatly across months and drainage events in 2020. In 2021, NO_3^- concentrations were very high in the early drainage events and rapidly declined in the latter.

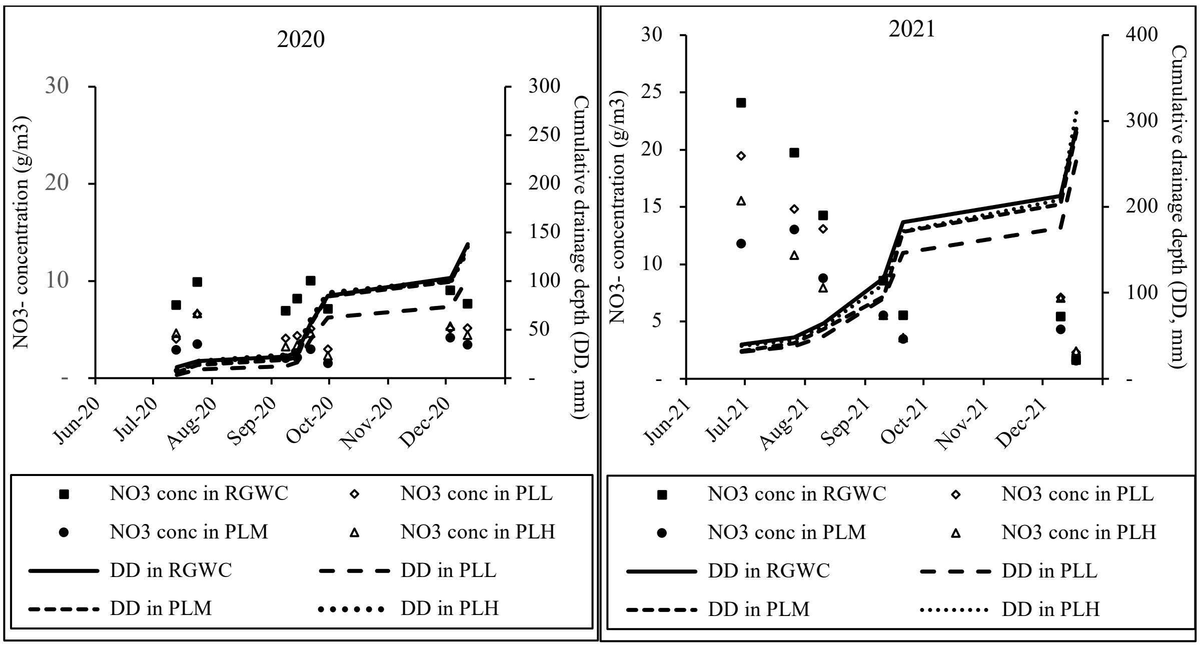
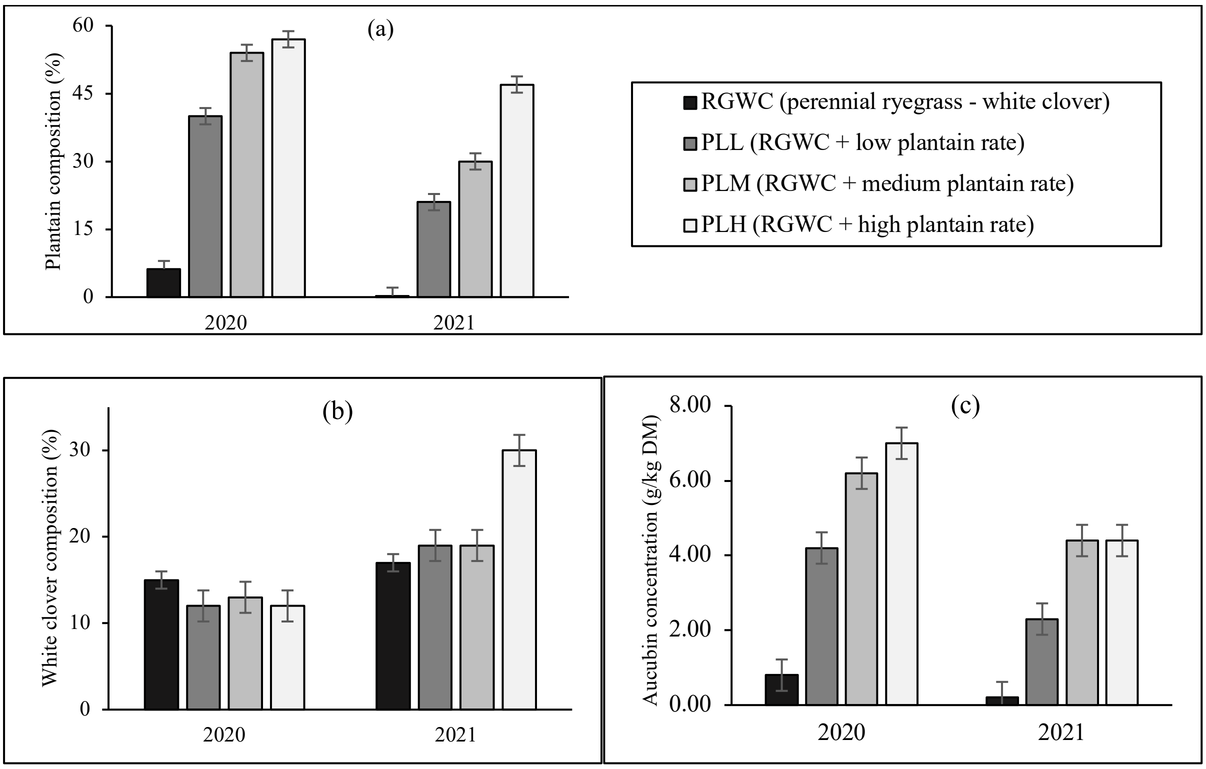


Figure 6.3. Drainage depth and nitrate (NO_3^-) concentration in drainage water from the pastoral dairy system with pasture treatments in 2020 and 2021.

Note: DD = drainage depth, perennial ryegrass-white clover (RGWC), RGWC + low plantain rate (PLL), RGWC + medium plantain rate (PLM), and RGWC + high plantain rate (PLH).

6.3.3. Herbage yield and composition, nitrogen uptake and fixation

The effect of pasture treatment on the plantain and white clover contents and the amount of BNF and N uptake by plants was different between the two experimental years (Figure 6.4, Appendix 7, $P < 0.05$). In 2020, white clover composition and BNF were similar across treatments ($P > 0.05$). However, the renovation of PLH in April 2021 resulted in a higher white clover content and BNF; additionally, herbage yield was lower, leading to a lower stocking density in PLH compared to other treatments in 2021 ($P < 0.05$). In 2020, PLH had a similar plantain content to PLM and tended to have higher herbage N uptake than other treatments. In 2021, the renovation resulted in much higher plantain and white clover contents and a far lower herbage N uptake on PLH than RGWC, PLL and PLM in 2021 ($P < 0.05$). On average, the plantain content in RGWC, PLL, PLM and PLH was 6, 40, 54 and 57% in 2020, and 1, 21, 30 and 47% in 2021, respectively ($P < 0.05$).



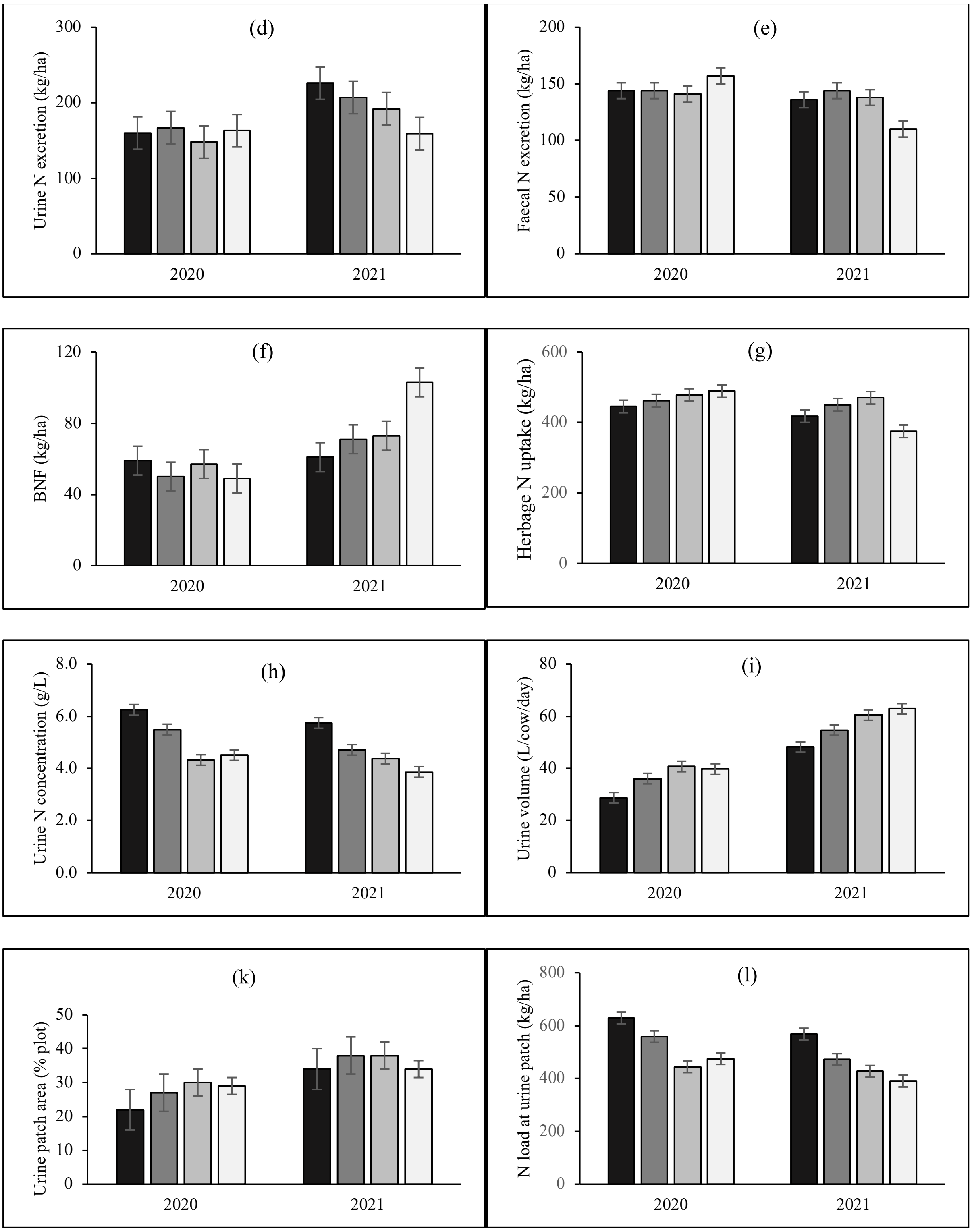


Figure 6.4. Plantain composition (a), white clover composition (b), aucubin concentration (c), urine N excretion (d), faecal N excretion (e), biological N fixation (BNF) (f), herbage N uptake (g), urine N concentration (h), urine volume (i), urine patch area (k), and N load at urine patch (l) of pasture treatments in 2020 and 2021.

Over the experimental period, herbage production ranged from 13,479–14,299 kg DM/ha/year and was similar across treatments (Table 6.3, $P > 0.05$). Plantain accounted for 3, 30, 42 and 52% of the sward in RGWC, PLL, PLM and PLH, respectively ($P < 0.05$). White clover composition in PLH was ~ 21% which was higher than the clover content in RGWC, PLL and PLM (~16%, $P < 0.05$). The concentration of aucubin was 0.55 in RGWC, 3.08 in PLL, 4.86 in PLM and 5.58 g/kg DM in PLH ($P < 0.05$). Annual herbage N uptake in PLL (456 kg DM/ha) and PLM (474 kg DM/ha) was higher than in RGWC (432 kg/ha/year) and PLH (432 kg/ha/year) ($P < 0.10$). The amount of N fixed by white clover ranged from 60–76 kg/ha/year, and there was no difference between treatments ($P > 0.05$).

Table 6.2. Characteristics of pasture and nitrogen (N) excretion and urine patches in the pastoral dairy system with pasture treatments over the experimental period.

Variable	RGWC	PLL	PLM	PLH[1]	SEM	P_T	P_Y	$P_{T \times Y}$
Pasture								
Annual herbage yield (kg DM/ha)	13,479	13,832	14,299	13,633	370	0.540	<0.001	0.052
Stock density (cow/ha)	2.6	2.6	2.6	2.3	-	-	-	-
Plantain composition (%)	3.3^a	30.3^b	41.9^c	50.2^d	1.29	<0.001	<0.001	<0.001
White clover (%)	16.0^a	15.6^a	16.3^a	20.7^b	1.25	0.023	<0.001	0.001
Aucubin (g/kg DM)	0.55^a	3.08^b	4.86^c	5.58^c	0.46	<0.001	0.002	0.775
Biological N fixed (kg N/ha/year)	60	61	65	76	7.3	0.199	<0.001	0.027
Herbage N uptake (kg N/ha/year)	432	456	474	432	12.6	0.064	0.003	0.018
Nitrogen excretion and urine patches								
Urine volume (L/cow/day)	38.5^a	45.4^b	50.6^c	51.3^c	1.46	<0.001	<0.001	0.648
N concentration in urine (g/L)	5.99^a	5.10^b	4.35^c	4.19^c	0.15	0.001	<0.001	0.184
Urinary N (kg N/ha/year)	190^a	185^a	170^b	162^b	14.7	<0.001	0.233	<0.001
Faecal N (kg N/ha/year)	139^a	144^b	144^b	134^c	4.9	<0.001	0.141	<0.001
Urine patch area (% paddock)	27.9^a	32.2^b	34.2^b	31.8^b	1.18	<0.001	<0.001	0.016
N load in patches (kg N/ha/year)	615^a	520^b	445^c	449^c	40.0	<0.001	0.319	0.239
Nitrogen leaching								
Nitrate leaching (kg/ha/year)	18.2^a	12.4^b	8.7^c	10.6^{bc}	1.23	<0.001	<0.001	0.084
Total N leaching (kg/ha/year)	27.6^a	19.5^b	17.9^b	18.7^b	1.36	0.001	<0.001	0.025

Note: [1] PLH was not grazed by dairy cows in May 2021 or June 2021 due to the renovation of this treatment, DM = dry matter, RGWC = perennial ryegrass-white clover, PLL = RGWC + low plantain rate, PLM = RGWC + medium plantain rate, PLH = RGWC + high plantain rate, SEM = standard error of the means, T = treatment, Y = year,

6.3.4. Nitrogen excretion and urine patches

There was a significant interaction between treatment and year in urine N and faecal N excretions (kg N/ha) and urine patch area (Figure 6.3, Appendix 7, $P < 0.05$). In 2020, urine and faecal N excretions were similar across treatments. However, PLH had lower estimated urine and faecal N excretion than RGWC, PLL and PLM in 2021 (Figure 6.3, $P < 0.05$). The lower urine and faecal N excretions in the PLH treatment in 2021 were due to the lower stocking density of this treatment (1.9 cows/ha) compared to other treatments (2.5 cows/ha). In addition, the effect of pasture treatment on urine patch areas in 2020 was higher than in 2021. Compared to RGWC, urine patch areas in PLL, PLM, and PLH were 23, 36 and 32% larger than in 2020, while PLL and PLM had a greater urine patch area than RGWC and PLH by 12% in 2021 (Figure 6.3, $P < 0.05$).

Over the experimental period, pasture treatment significantly affected urine N concentration, urine volume, urine and faecal N excretions, urine patch area, and N load at urine patches (Table 6.3, $P < 0.05$). Cows grazing the PLL, PLM and PLH treatments had a greater urine volume by 17, 31 and 33% and a lower urine N concentration by 15, 27 and 30%, respectively, than those grazing RGWC. In addition, UN excretion (kg N/ha) in PLM and PLH was lower than in RGWC and PLL (Table 6.3, $P < 0.05$), while faecal N excretion in PLL and PLM was higher than in RGWC and PLH ($P < 0.05$). Furthermore, PLL, PLM and PLH had a larger area covered with urine patches by 15, 23 and 14%, a lower N load in urine patches by 14, 28 and 27%, respectively, compared to RGWC (Table 6.3, $P < 0.05$).

6.4. Discussion

To available knowledge, the present study is the first measurement of the relationship between plantain content in the sward and the quantity of NO_3^- leaching from a pastoral dairy system at the plot scale. The results of the current study indicated that incorporating 30–50% plantain in grazing swards could be a successful strategy to significantly reduce NO_3^- leaching from pastoral dairy systems. The increased urine patch area, the decreased N load at urine patches and the greater N uptake by pasture are likely the main drivers of the reduced NO_3^- leaching under plantain-based pastures. Furthermore, the greater reduction in NO_3^- leaching under the medium and high plantain content treatments compared to under the low plantain content treatment, along with the decrease in plantain content in all treatments from the second year, suggest that there is a need to explore management practices to maintain the proportion of plantain beyond two years from establishment if NO_3^- leaching is to be mitigated in the longer term.

The average annual NO_3^- leached in the present study, between 8.7 and 18.2 kg per ha, was in the range of NO_3^- leaching measured from pastoral dairy systems on Tokomaru silt loam soil (Christensen et al., 2018; Rodriguez et al., 2020). Furthermore, the majority of NO_3^- leaching occurred during the early drainage season (from June-September or the first 100 mm of drainage) (Christensen et al., 2018). According to Di and Cameron (2002), most of the NO_3^- which leaches from pastoral soils is from the cow urine deposited during late summer and autumn when the pasture growth rate is low, and the N load in urine patches exceeds the N uptake by pasture. As a result, NO_3^- in urine patches can result in drainage with a high NO_3^- concentration when heavy rains occur in early winter. On the other hand, less NO_3^- is leached during spring and early summer because the rapid herbage growth rate allows the pasture to uptake most N in urine patches over this period (Christensen et al., 2018; Houlbrooke et al., 2003). Interestingly, in 2020, the total amount of NO_3^- leached was small, and the concentration of NO_3^- was low, even in the early drainage events in winter. This low leaching is likely due to the low drainage volume in this dry year, so even though an excess of NO_3^- may have been present in the soil, not much NO_3^- ended up being leached (Jabloun et al., 2015).

The reduction of 32–52% in NO_3^- leaching under the plantain-based pastures in the present study was almost equivalent to the 48% reduction measured by Rodriguez et al. (2020) in a study comparing NO_3^- leaching from pure plantain versus RGWC. However, both of these results are far lower than those of Woods et al. (2018) and Carlton et al. (2019), who reported a 74–89% reduction in NO_3^- leaching by incorporating plantain with traditional grasses. However, these studies used relatively small lysimeters. The smaller decrease in NO_3^- leaching observed at the large plot scale in the present study could be related to the relative areas covered by urine patches which are considered the primary source of NO_3^- leaching (Di & Cameron, 2002). Plantain-based pastures likely have a greater N uptake from urine patches due to the greater patch area. This, in turn, will reduce NO_3^- leaching compared with RGWC with smaller, more concentrated N urine patches (Selbie et al., 2015). The estimated area covered by urine patches in the present study was approximately 30% annually, while N was applied at an equivalent rate to urine patches to the whole area in the lysimeter studies (Carlton et al., 2019; Woods et al., 2018).

The reduction in NO_3^- leaching was lower in the second year (2021) compared to the first year (2020) in the present study. This difference may relate to the content of plantain in the swards. The average plantain content of PLL, PLM and PLH was 50% in 2020 and 33% in 2021, resulting in a 62% and 33% reduction in NO_3^- leaching in 2020 and 2021, respectively. Plantain content in the sward has been shown to influence urine production and UN excretion of dairy cows (Minnée et al., 2020; Nguyen et al., 2023), and both of these factors can affect NO_3^- leaching (Ledgard et al., 2015; Romera et al., 2012). While all PLL, PLM and PLH resulted in a significant reduction in NO_3^- leaching, the medium (PLM) and high (PLH) plantain content treatments showed a greater decrease in NO_3^- leaching than the low (PLL) plantain content in at least the two years after establishment. These higher plantain content treatments (PLM and

PLH) also had no adverse effects on annual pasture yield (as in Chapter 3 and by Nguyen et al. (2022b)) or milk production (as in Chapters 4–5, and by Nguyen et al. (2023)).

The reduction in NO_3^- leaching suggests that incorporating plantain, at a proportion of at least 30% in the pastures, with RGWC is a promising option to mitigate NO_3^- leaching from pastoral dairy systems. Plantain integrated with RGWC could result in a greater reduction in NO_3^- leaching from pastoral systems in a more extended period than when it is sown as a monoculture or mixed with red clover. Rodriguez et al. (2020) reported that a pure plantain sward only reduced NO_3^- leaching for the first year, while a plantain-clover mix only decreased NO_3^- leaching in the establishment year (first half year). The main reason for this could relate to the resilience of plantain in the pastures. In grazed mixed swards, plantain can establish rapidly after sowing and grow well in the first year. However, ryegrass and clover will become dominant in the second year (Dodd et al., 2019; Nguyen et al., 2022b). In pure plantain or plantain-clover mixes, weeds and clovers often outcompete plantain after a year from the establishment, strongly impacting the survival of plantain in swards (Gawn et al., 2012; Rodriguez et al., 2020). Compared with Rodriguez et al. (2020), the plantain content in the present study was lower in the first year but higher in the second year. In addition, the clover content in the plantain-RGWC pasture in the present study was lower than that in the pure plantain and plantain-clover mixes in Rodriguez et al. (2020). The higher clover content means more biological N can be fixed, increasing the total N input to the system and the risk of NO_3^- leaching (Ledgard et al., 2009). The contrasting effect of plantain and clover on NO_3^- leaching highlights the need to identify the balance between plantain, clover and ryegrass in pastures if greater environmental benefits and farm productivity are to be achieved.

The reduction in plantain content in the PLH treatment beyond two years after establishment suggests a need to investigate management practices that maintain the percentage of plantain and its ability to mitigate NO_3^- leaching in the longer term. From early 2021, plantain content in PLH decreased to match that in PLM, requiring the PLH treatment to be renovated in an attempt to increase plantain content in the sward. Although the intervention notably increased plantain content, it did not successfully further reduce NO_3^- leaching from PLH this year. After removing perennial ryegrass, the existing plantain and clover rapidly become the dominant species, resulting in a significantly higher plantain and white clover content in PLH than in PLM in the second year. The higher clover content could increase NO_3^- leaching from this treatment, as more biological N was fixed, which increased the total N input to the system (Ledgard et al., 2009; Lucas et al., 2010). In addition, herbage production of PLH was lower than other treatments because the renovation resulted in less N uptake by ryegrass in winter (Corré et al., 2014; Di & Cameron, 2002). The high NO_3^- leaching from PLH in 2021 could also have been impacted by killing the resident ryegrass and disturbing the soil by oversowing. Further studies are necessary to develop the management practices to maintain the advantages of the RGWC pastures with a large plantain component (at least 30%) or to reduce at least 30% NO_3^- leaching beyond two years from establishment.

The effect of plantain-based pastures on reducing NO_3^- leaching is related to various N processes within the animal-plant-soil system. For animals, including plantain in the cow diet increases urine production and reduces UN concentration (Mangwe et al., 2019), spreading UN over a larger area of urine patches, resulting in urine patches with lower N load (Romera et al., 2012). Also, grazing plantain-based pastures decreases the UN excretion of dairy cows due to improving N utilisation for milk production and partitioning more N to faeces (Marshall et al., 2021; Minnée et al., 2020; Navarrete et al., 2022). For the pasture, the larger urine patch area allows pastures to uptake more, or at least a greater proportion, of N from the patch (Bryant, Snow, et al., 2019; Cichota et al., 2018; Judson et al.; Woods et al., 2018). More N partitioned to faeces means that more of the total N is in a less-soluble form and, therefore, not as readily leached (Petersen et al., 1998). In the soil, plant bioactive compounds such as aucubin released from plantain roots could inhibit the nitrification process that transforms N in urine and faeces to NO_3^- and N_2O, thereby reducing both NO_3^- leaching and nitrous oxide emission (Cameron et al., 2013; de Klein et al., 2019; Di et al., 2010). In the present study, incorporating an average of 41% plantain into a RGWC pasture over two years contributed to 9% decrease in UN excretion, a 17% increase in urine patch area, a 23% decrease in UN load, and a 5% increase herbage N uptake, compared with the RGWC pasture. The presence of 3.08–5.58 g aucubin/kg DM in the plantain-based pasture may explain improved animal N utilisation and the possible nitrification inhibition in the soil. The combination of increasing urine patch area and reducing urine N excretion can explain the increase in N uptake and the reduction in NO_3^- leaching in 2021. However, the dilution of N in greater urine volume is likely the main driver of the decrease in NO_3^- leaching in 2020. Perhaps other factors, such as weather conditions and grazing management, may influence the impact of plantain content on urine production, urine N concentration and urine N excretion (Box et al., 2017; Gregorini et al., 2018). These factors were not considered in the rationale of the present study and are required to be addressed in further research.

The results presented above suggest that incorporating plantain into the RGWC pasture successfully achieved 30–50% plantain, which resulted in a reduction of ~30–50% NO_3^- leaching over two years from establishment. However, obtaining 70% plantain or achieving a 70% reduction in NO_3^- leaching, as the hypothesis tested for the high plantain content treatment, is likely impossible when plantain is integrated with RGWC in grazing swards, even with a high plantain seed rate at sowing. Compared to PLL, both PLM and PLH resulted in higher contents of plantain and concomitant reductions in NO_3^- leaching while having no adverse effect on pasture yield (as in Chapter 3 and by Nguyen et al. (2022b)), milk yield or milk composition (as in Chapters 4–5 and by Nguyen et al. (2023)) relative to RGWC. A lower plantain seeding rate resulted in a smaller plantain content in the sward and a lower reduction in NO_3^- leaching from the pastoral system. In contrast, sowing with a very high plantain seeding rate can reduce the resilience of plantain over an extended period. The reasons for this may relate to the natural balance of ryegrass-clover-plantain in the mixtures. According to Dodd et al. (2019), ryegrass-based pasture under grazing conditions often has less than 20% after three years from sowing. This suggests that all the treatments may have 30% or less plantain in the long term, even those with high plantain contents in the first and second years. Another possible driver could be due to the lower residual of the high plantain content treatments compared to the low plantain content treatment. Although the relationship between post-grazing height and the persistence of plantain in mixed swards is unknown, the observation from the present study found that overgrazing could increase the damage of plantain and reduce its recovery. At the same time, more available spaces due to overgrazing allow ryegrass, clover and weeds to develop from the second year onward (Bryant, Dodd, et al., 2019; Stewart, 1996). For example, when ryegrass was removed and plantain was oversown in the PLH plots, plantain content increased rapidly in the two subsequent grazings, but it then reduced dramatically in the following grazings. The replacement of plantain with white clover in the sward is likely the main reason for increasing NO_3^- leaching from this treatment in the second year. In addition, the higher percentage of plantain in PLH in the first year did not result in a greater reduction in NO_3^- leaching than from PLM. Together with the results of Rodriguez et al. (2020), the present study suggests that balancing the compositions of ryegrass-clover-plantain is likely more important to the reduction in NO_3^- leaching than having a very high plantain content. In practice, traditional RGWC often contains around 10–20% clover (Soegaard, 2009). This clover content already exceeds the N requirement of lactation cows; therefore, a higher clover content will increase UN excretion and NO_3^- leaching (Di & Cameron, 2002; Eriksen et al., 2004). An average of 30 or 50% plantain is a practical target proportion that led to a 32% and 52% reduction in NO_3^- leaching, respectively. Beyond two years from establishment, plantain at all sowing rates is expected to comprise 30% or less as a proportion of the whole sward, possibly lowering the effect of pasture treatments on reducing NO_3^- leaching (Dodd et al., 2019; Nguyen et al., 2022b). Therefore, the effectiveness of the pasture treatments in the present study needs to be measured in the longer term or identification of management practices to extend the advantages of plantain-based pastures in reducing NO_3^- leaching.

6.5. Conclusion

Incorporating an average of 30 and 50% plantain with RGWC decreased NO_3^- leaching from the pastoral dairy system by 42 and 52% over two years from establishment. The reduction in NO_3^- leaching was greater in the first year than in the second year, associated with the plantain content in the pasture. The decrease in NO_3^- leaching was associated with larger urine volume, lower UN concentration, enlarged urine patch area, and consequently, lower N load at urine patches. Increasing N uptake by pasture on plantain treatments may also have been a factor. These results suggest that plantain-RGWC pasture could be a promising strategy to mitigate NO_3^- leaching from pastoral dairy systems. However, management practices need to be explored beyond two years after establishment if NO_3^- leaching is to be reduced in the longer term.

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CHAPTER 7

OVERALL DISCUSSION

The objectives of this thesis were to address, throughout the four result chapters, the effect of the plantain-based pastures on:

- pasture production in Chapter 3
- urine volume and frequency in Chapter 4,
- milk production and urinary nitrogen excretion in Chapter 5, and
- nitrate leaching in Chapter 6.

Chapter 7, an overall discussion, integrates all the findings into a comprehensive summary, discusses the research outcomes, highlights practical implications and recommends further work to improve the use of plantain-based pasture.

7.1. Overall summary

The present study aimed to quantify the impact of plantain (*Plantago lanceolata*) (cv. Agritonic) incorporated at increasing rates with perennial ryegrass (*Lolium perenne*) (cv. ONE^{50})-white clover (*Trifolium repens*) (cv. Tribute) (RGWC) on farm productivity and environmental benefit. A field grazing trial was conducted on a 7-ha paddock over 36 months, comprehensively measuring pasture and milk production, nitrogen (N) excretion, and nitrate (NO_3^-) leaching from the pastoral system. The outcomes of the research are presented and discussed in Chapter 3-6. The main findings are demonstrated in Figure 7.1 and summarised below:

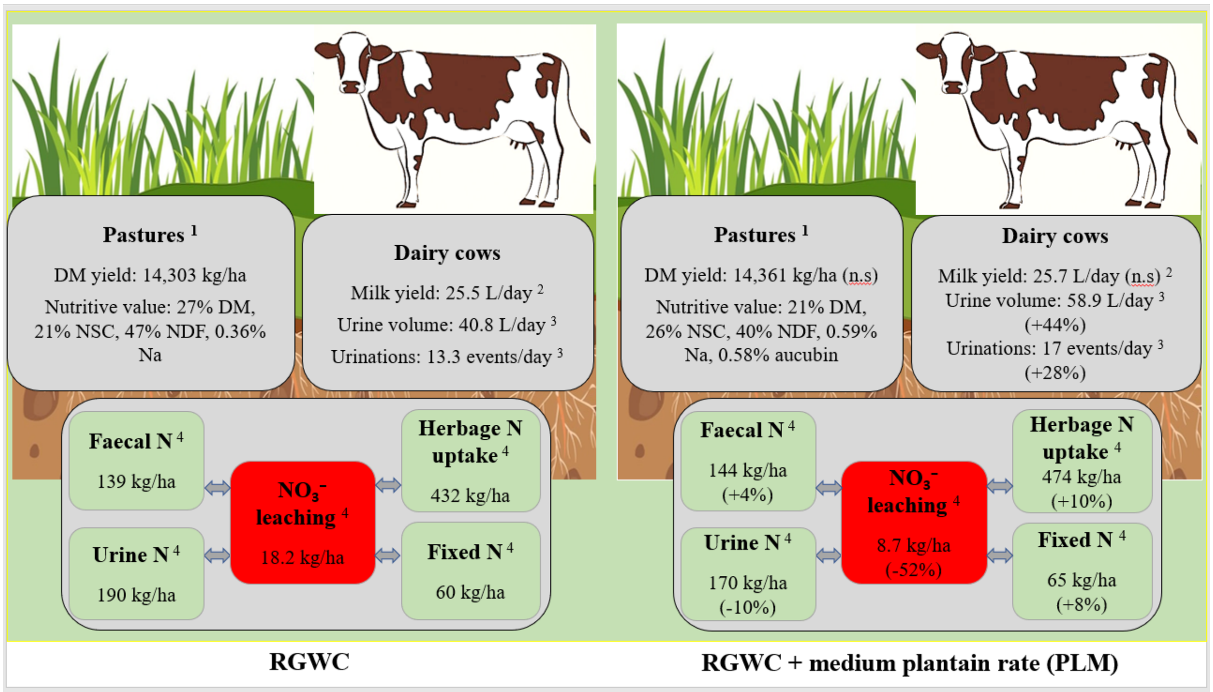

Figure 7.1. Pasture production, milk yield and nitrate (NO_3^-) leaching from the pastoral dairy systems with perennial ryegrass-white clover (RGWC) and with RGWC + medium plantain rate over the experimental period.

Note: [1] data on pasture production were obtained from the first grazing to day 705 or from September 2019 to March 2021 (Chapter 3), [2] milk yield was the mean value from all 14 grazings over two lactation seasons between September 2019 to March 2021 (Chapter 5). [3] data on urine volume and urination frequency were measured using sensors from two grazings in March 2021 and May 2021 (Chapter 4). [4] data on NO_3^- leaching were measured for two drainage leaching seasons between January 2020 and December 2021.

- **Chapter 3** showed that incorporating plantain into RGWC pasture maintained herbage yield. Sowing with low, medium, and high rates of plantain seeds with RGWC achieved an average of 32, 44, and 48% plantain (including leaves and reproductive stem) in the first 705 days from sowing, respectively. The plantain content increased during the first 15 months, but then declined rapidly to about 20–30% at day 705. In addition, plantain incorporation improved organic matter digestibility, non-structural carbohydrates, phosphorus, sulfur, magnesium, sodium, chloride, zinc, boron, cobalt, aucubin, acteoside, and catalpol, while reducing dry matter content, acid detergent fibre, neutral detergent fibre, crude fat, iron, and manganese. These changes were linearly associated with plantain content and were more significant in late summer and autumn than in spring and early summer.
- **Chapter 4** demonstrated that including 25% plantain in the diet resulted in a 44% increase in daily urine volume and a 28% increase in urination frequency compared to the diet without plantain. Furthermore, cows that consumed 25% dietary plantain had a 29% lower urine nitrogen concentration than those fed without plantain. When dietary plantain was reduced to 18%, there was only a small effect on increased urine volume, though urine N concentration was still reduced by 18%.
- **Chapter 5** showed no significant difference in milk yield or milk solids production or in the proportions of protein, fat and lactose in milk from cows grazing the pastures with and without plantain. However, incorporating an average of 23% plantain in the diet reduced UN concentration by 22% and UN excretion by 7% while increasing urine volume by 33%. The effects of the plantain-based pastures on urine N concentration, UN excretion and urine volume were associated with the proportion of plantain in the diet and were greater in late summer and autumn than in spring and early summer.
- **Chapter 6** demonstrated that incorporating an average of 30 and 50% plantain with

RGWC decreased NO_3^- leaching by 32 and 52%, respectively, over two drainage years after the establishment of the pasture, with a greater reduction in the first year than in the second year due to plantain content declining. Among treatments, PLM provided the most significant decrease in NO_3^- leaching. This treatment had an average of 54% plantain in 2020 and 30% plantain in 2021, resulting in 66 and 46% lower NO_3^- leaching, respectively, compared to RGWC. The decrease in NO_3^- leaching was associated with greater urine volume, lower UN conconcentration, larger urine patch area with smaller N load, andhigher N uptake by plants.

7.2. Main findings and practical implications

7.2.1. Reducing nitrate leaching

The main objective of incorporating plantain with RGWC pasture is to reduce NO_3^- leaching from pastoral dairy systems, as indicated in lysimeter studies (Carlton et al., 2019; Woods et al., 2018) or modelling work (Al-Marashdeh et al., 2021; Doole et al., 2021). Chapter 6 of this thesis was the first measurement of the impact of plantain-based pasture on NO_3^- leaching at the paddock level. Compared to lysimeter experiments, the reduction of NO_3^- leaching was lower at the paddock scale when plantain was included in the pasture. This could relate to the lower UN load and coverage area of urine patches at the paddock compared to the lysimeter studies. However, the 32–52% reduction in NO_3^- leaching during the experimental period suggests that plantain-based pasture can be an effective option for farmers to mitigate N losses from pastoral dairy farms for at least two years from the establishment of plantain. The research also revealed that the reduction in NO_3^- leaching is associated with the N cycling in the pasture-animal-soil system at the plot scale, including increasing urine volume and UN concentration of dairy cows, enlarging urine patch area with smaller N load, and increasing N uptake by pasture.

7.2.2. Need of management beyond two years

Managing at least 30% on a DM basis in swards is desirable to mitigate NO_3^- leaching by 30% or more from dairy farms consistently in the longer term. In the present research, an average of 30% plantain (the low plantain content treatment (PLL)) reduced NO_3^- leaching by 32% over two years from establishment. However, the plantain proportion declined rapidly to 20% in PLL and 30% in the medium (PLM) and high (PLH) plantain content treatments at day 705 from sowing and is expected to account for 30% or less of the total DM in mixed pastures from year three from establishment (Dodd et al., 2019). These results show that management is likely required beyond two years from the establishment of plantain to achieve more than 30% plantain or a concomitant 30% reduction in NO_3^- leaching at the whole-farm level. Periodically renewing plantain-based pastures with medium to high plantain sowing rates (7–10 kg plantain seed/ha) could be an effective option to maintain sufficient plantain content over the whole farm to facilitate a significant decrease in N leaching. However, more research is needed to determine a suitable pasture renewal area to meet specific targets for reduced N losses.

7.2.3. Maintaining farm productivity

In addition to the benefits of reducing NO_3^- leaching from pastoral systems, incorporating plantain into the RGWC pasture was hypothesised to maintain or increase farm productivity, as presented in previous short-term studies (Al-Marashdeh et al., 2021; Woods et al., 2018). Chapters 4–6 of this thesis indicated that plantain incorporation maintained pasture production and milk yield under the conditions of the present study; however, neither were these decreased. The results of this thesis, which show that farm productivity was maintained whilst NO_3^- leaching from the pastoral system was reduced, should provide confidence to farmers to implement plantain into their systems.

While the present research indicated that incorporating plantain with RGWC pasture at least maintained herbage yield and milk production of dairy cows, it is expected that in drier environments, or under conditions of drier summers than those experienced in the present study, the heat and drought tolerance of plantain would increase pasture production (Cranston et al., 2016). In addition, the higher nutritional quality of plantain compared with RGWC under dry conditions might improve milk production of cows (Dodd et al., 2018). At the national scale, the model of Doole et al. (2021) estimated an economic benefit of \$40 to \$177 per ha to the New Zealand dairy sector, resulting both from increased milk production and decreased NO_3^- leaching, when plantain was integrated into 15–45% of the farm area.

7.2.4. Practical implications

The results of the present thesis provided several practical implications for improving the use of plantain on farms:

- Firstly, incorporating plantain with RGWC pasture could provide better results than pure plantain and plantain-clover mixes in reducing NO_3^- leaching from pastoral dairy systems and for the persistence of plantain in grazing swards. For instance, in the present study, the plantain-based pastures resulted in significantly lower NO_3^- leaching than the RGWC pasture over the two measured years. However, compared to RGWC pasture, NO_3^- leaching from a pastoral dairy system was only statistically lower in pure plantain during the establishment period (8 months from sowing) and in the plantain-clover mix during the first year (a full year after the establishment period) in the study of Rodriguez (2020). In addition, sowing plantain with RGWC can maintain a better balance between plantain, ryegrass, and clover than sowing plantain either as a monoculture or with a companion legume. The presence of ryegrass may control the compositions of clover and weed in the plantain-based pasture to the same level as in the RGWC pasture if no intervention is applied (Nguyen et al., 2022). In contrast, clover and weed can become dominant species after one or two years from establishment when plantain was sown either as a monoculture or in a mixture with clover (Gawn et al., 2012). Therefore, the maintenance of ryegrass and clover content in mixed swards with plantain will not only guarantee the yield and quality of the pasture but also mitigate the adverse effect of high clover content on increasing NO_3^- leaching from pastoral systems in the long term (Rodriguez, 2020).

- Secondly, the sowing rate of the PLM treatment provided the most stable plantain content of the three treatments, along with the most significant reduction in NO_3^- leaching for an extended period. In the PLH treatment, the renovation in April 2021 caused this treatment to have a different effect from the other treatments. Therefore, no conclusion can be made by comparing PLH and PLM over the experimental period. However, the two treatments were similar in pasture production, plantain content, milk production, UN excretion and NO_3^- leaching in the first year prior to the renovation intervention. Therefore, PLH is expected to have a similar, or not much different effect, on-farm production and NO_3^- leaching compared to PLM.
- Additionally, the renovation for PLH suggests that removing ryegrass and resowing new seeds after two years from sowing is unsuccessful in reducing NO_3^- leaching or maintaining farm productivity. This intervention increased the percentage of plantain for a short period and also increased clover and weed contents from the subsequent grazing onwards. Consequently, PLH had lower pasture production and higher NO_3^- leaching than PLM in the second year.

7.3. Recommendations for future research

The results of this thesis provide reliable evidence of the effect of plantain-based pasture on significantly reducing NO_3^- leaching while maintaining farm productivity. The thesis also provides practical advice to improve the use of plantain in pastoral systems. However, several questions require addressing in further studies:

- Firstly, the effect of plantain-based pastures should be determined in the longer term. The present thesis measured the impact of experimental pastures over 36 months from sowing and indicated that plantain incorporation reduced NO_3^- leaching over the two measurement years where leaching was recorded. However, plantain content declined to 20–30% of total pasture DM at the end of year two and was expected to continue to decline in subsequent years. This reduction in plantain content raises a question: "Is plantain content lower than 30% effective in reducing NO_3^- leaching, or will additional management be needed to increase plantain content to maintain its effectiveness at a paddock level in further years?". To answer this question, further measurements of pasture yield and nutritive value, the persistence of plantain, milk production and UN excretion of dairy cows, and NO_3^- leaching should be conducted until no significant effect of the plantain-based pastures is found.
- Secondly, further studies are required to investigate management strategies to maintain the proportion of plantain in grazing pastures beyond two years from establishment. The present thesis indicated that 30% plantain reduced NO_3^- leaching from the pastoral system by 32%. Therefore, if more than a 30% reduction in NO_3^- leaching is targeted, maintaining plantain content to at least 30% and probably applying different mitigation strategies will be necessary. This thesis showed that removing ryegrass and oversowing new plantain seeds was unsuccessful in reducing NO_3^- leaching in the year it was trialled. In addition, Bryant et al. (2019) reported that direct drilling and broadcasting plantain seeds into existing pastures are unlikely to achieve 30% or more plantain in the sward. Therefore, alternative management strategies are required to obtain a higher plantain content and concomitant lower NO_3^- leaching for more than two years from

establishment. Several possible approaches could be considered, including the timing of grazing to limit treading damage, the renovation of pasture on farms after two or three years, exploration of plantain-specific grazing management, including suitable timing and intensity of grazing, or exploring alternative management.

- Thirdly, there is a need to fully understand the mechanism by which plantain influences N recycling in the soil-pasture-animal system. Several mechanisms have been investigated in previous studies. For example, a balance between soluble energy and protein in herbage, along with the presence of secondary compounds in plantain, can modify the use of dietary N by animals and reduce UN excretion (Navarrete et al., 2016), and this, along with the inhibition of nitrification processes in soils, acts to decrease NO_3^- leaching (Podolyan et al., 2019). However, the contribution of each factor to UN excretion and NO_3^- leaching at the paddock level has not been identified. Plantain has also been reported to mitigate nitrous oxide emissions from pastoral systems (Luo et al., 2018; Simon et al., 2019) and methane emissions from dairy cows (Della Rosa et al., 2022). Measurement of the potential of plantain-based pasture on nitrous oxide and methane emissions and understanding how plantain reduces greenhouse gas emissions are necessary to extend the benefit of this strategy at the farm scale.
- Additionally, plantain must be integrated into agriculture system models to give farmers and authorities reliable estimations of its effect. However, with limited data, it is difficult to produce highly accurate models. Therefore, more field research will be needed to improve these estimations. Moreover, alternative methods to determine plantain content on farms should be developed so farmers can quickly identify plantain content conveniently.
- Finally, additional measurements are necessary to assess the impact of plantain-based pastures on different soil types, environments, and farming practices. The duration of

urine N retention in the soil is influenced by soil texture and the amount of rainfall, which, in turn, affects the effectiveness of plantain-based pastures in reducing NO_3^- leaching (Carlton et al., 2018; Eriksen et al., 2004). The current study was conducted on extensive dairy farms with Tokomaru silt loam soil during a relatively low rainfall period. By measuring the effects of plantain-based pastures in other systems with varying soil types and locations, farmers can gather more evidence before implementing this technology on their own farms.

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APPENDICES

Appendix 1. A review article supporting for Chapter 2

Title: Forage plantain (*Plantago lanceolata* L.): Meta-analysis quantifying the decrease in nitrogen excretion, the increase in milk production, and the changes in milk composition of dairy cows grazing pastures containing plantain

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Highlights

- Pastures containing plantain decrease UN excretion by dairy cows.
- The decrease in UN excretion is associated with plantain content.
- Pastures containing plantain increase milk yield in late lactation.
- Plantain inclusion reduces milk fat concentration but maintains milk fat yield.

Abstract

Plantain (*Plantago lanceolata* L.) has been increasingly used as a forage component in dairy grazing systems due to its capacity to reduce N losses, while improving or maintaining milk production. A meta-analysis was conducted to quantify the effect of plantain on milk production and UN excretion by dairy cows. The main outcomes of this analysis included the yield, solids (fat + protein), protein and fat components of milk, and N concentration in urine, daily urine volume, and total UN excretion by dairy cows. Overall, grazing pastures containing plantain significantly increased milk yield (weighted mean difference (WMD) = 1.02 kg/cow/day, 95% confidence interval (CI) = 0.55 to 1.46), milk solids yield (WMD = 0.07 kg/cow/day, 95% CI = 0.02 to 0.12), and milk protein yield (WMD = 23.4 g/cow/day, 95% CI = 11.3 to 35.5), maintained milk protein concentration and milk fat yield, but reduced milk fat concentration (WMD = -0.24%, 95% CI = -0.31 to -0.17). Feeding pastures containing plantain reduced total UN excretion by 22% (95% CI = 15 to 28), which was associated with a decrease of 30% in UN concentration (95% CI = 20 to 38) and an increase of 17% in daily urine volume (95% CI = 7 to 29). Subgroup analysis showed that cows grazing pastures containing plantain had a significantly higher milk yield in late lactation (WMD = 1.4 kg/cow/day, 95% CI = 0.8 to 1.9), but a similar milk yield in early lactation, compared to grazing control pastures. In addition, meta-regression analysis found statistical associations between the content of plantain in the diet and N concentration in urine ($P < 0.001$), daily urine volume ($P < 0.001$), and total UN excretion ($P = 0.036$). The results suggest that incorporating plantain into grazing pastures is a potential strategy for improving farm productivity, while reducing the environmental impact of dairy farms.

Keywords: grazing swards, milk yield, plantain forage, urinary nitrogen, urine volume.

1. Introduction

Plantain (*Plantago lanceolata* L.) is a perennial forage herb, which has been increasingly used in the last decade as a special purpose crop, or a component of pasture mixes for dairy cattle in temperate countries such as New Zealand, Australia, and Ireland. Forage plantain is tolerant to drought and heat (Cranston et al., 2015; Stewart, 1996), and produces more dry matter (DM) than RGWC in summer and autumn in dryland pastoral systems (Moorhead & Piggot, 2009). Sole plantain contains 110 g/kg DM higher non-structural fibre, 26 g/kg DM lower soluble N in N content (Minneé et al., 2019), and 1.5 times greater macro minerals (Mangwe et al., 2019) than RG. Furthermore, it has a high concentration of secondary compounds, aucubin and acteoside (Navarrete et al., 2016) that are known to not exist in ryegrass and white clover.

Intensive studies have suggested that plantain as a pure sward (Box et al., 2017; Mangwe et al., 2019) or combined in a mixed pasture reduces N excretion from cow urine, while maintaining or increasing milk production. Feeding pastures containing plantain reduces N concentration in cow urine, and increases urine volume and frequency (Mangwe et al., 2019; Minnée et al., 2020); therefore, potentially decreasing N leaching from dairy farms (Bryant et al., 2019). Other studies have shown the benefit of plantain forage in improving milk yield (McCarthy et al., 2020) and altering the fatty acid profile

(Mangwe et al., 2018). However, the effects of plantain on N excretion and milk production are highly variable across studies. For example, pasture containing plantain can reduce UN excretion from 0 (Minnée et al., 2020) to 44% (Mangwe et al., 2019), while maintaining (Nkomboni et al., 2021) or increasing milk yield (Totty et al., 2013). This raises the questions "to what extent does incorporating plantain in grazing pastures affect N excretion and milk production of dairy cows?" and "how are the effects influenced by experimental conditions such as lactation stage and the content of plantain in the diet?".

The present review paper used meta-analysis to compare milk production and UN excretion by dairy cows offered either pasture containing plantain (treatment pasture) or not (control pasture). Additionally, the effects of plantain at different lactation stages and the effect of varying plantain content in the diet were compared.

2. Materials and Methods

This meta-analysis was conducted in compliance with the method described in "Cochrane Handbook for Systematic Reviews of Interventions" (Higgins et al., 2021) and the statistical analysis introduced in "Doing Meta-Analysis in R" (Harrer et al., 2021). The review questions were: (1) to what extent does the use of plantain in grazed pastures affect milk production and UN excretion by dairy cows? And (2) how are the effects of plantain different over stages of lactation and with varying contents of plantain in the diet of dairy cows?

2.1. Literature search

Online search engines, Web of Science (http://webofknowledge.com), and Google Scholar (https://scholar.google.com) were used in the literature search to obtain relevant studies. Keywords for searching were "plantain" AND "dairy cows". The reference lists from publications were reviewed to find more studies. Moreover, a 'connected papers graph' was created and used https://www.connectedpapers.com, to obtain a visual overview to double-check against the list of studies produced by the literature search (Figure A1.1).

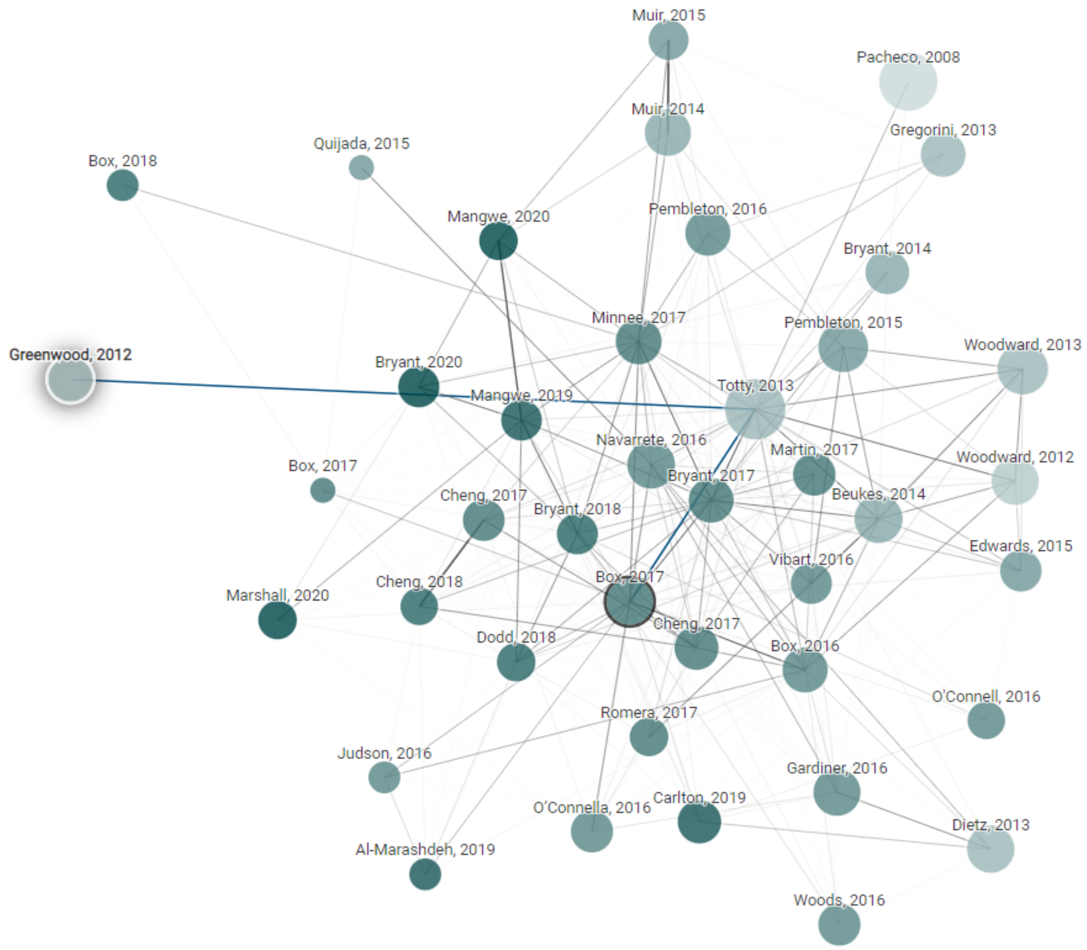


Figure A1.1. Papers published from 2012–2021 connected with Box et al. (2017) on milk production and N excretion of dairy cows grazing pastures containing plantain.

2.2. Publication selection and selection criteria

The literature search and study selection processes are presented in Figure A1.2. A total of 134 studies was obtained from the literature search. In which, 122 were excluded by title and abstract screening (105) and full manuscript scanning (17). A total of 12 studies from New Zealand (11) and Australia (1) were selected for this review. All selected studies (i) contained a full manuscript in English, (ii) were conducted on lactating dairy cows using at least a treatment pasture and a control pasture, (iii) demonstrated a clear experimental design, (iv) had at least one comparison for milk production or UN excretion, and (v) presented at least one measure of the statistical variance of the comparisons (standard deviation, standard error, or P-value). Studies with pastures containing other herbs or grasses, with known potential to greatly affect milk production or N excretion, such as chicory (Cichorium intybus L.), were excluded in this meta-analysis.

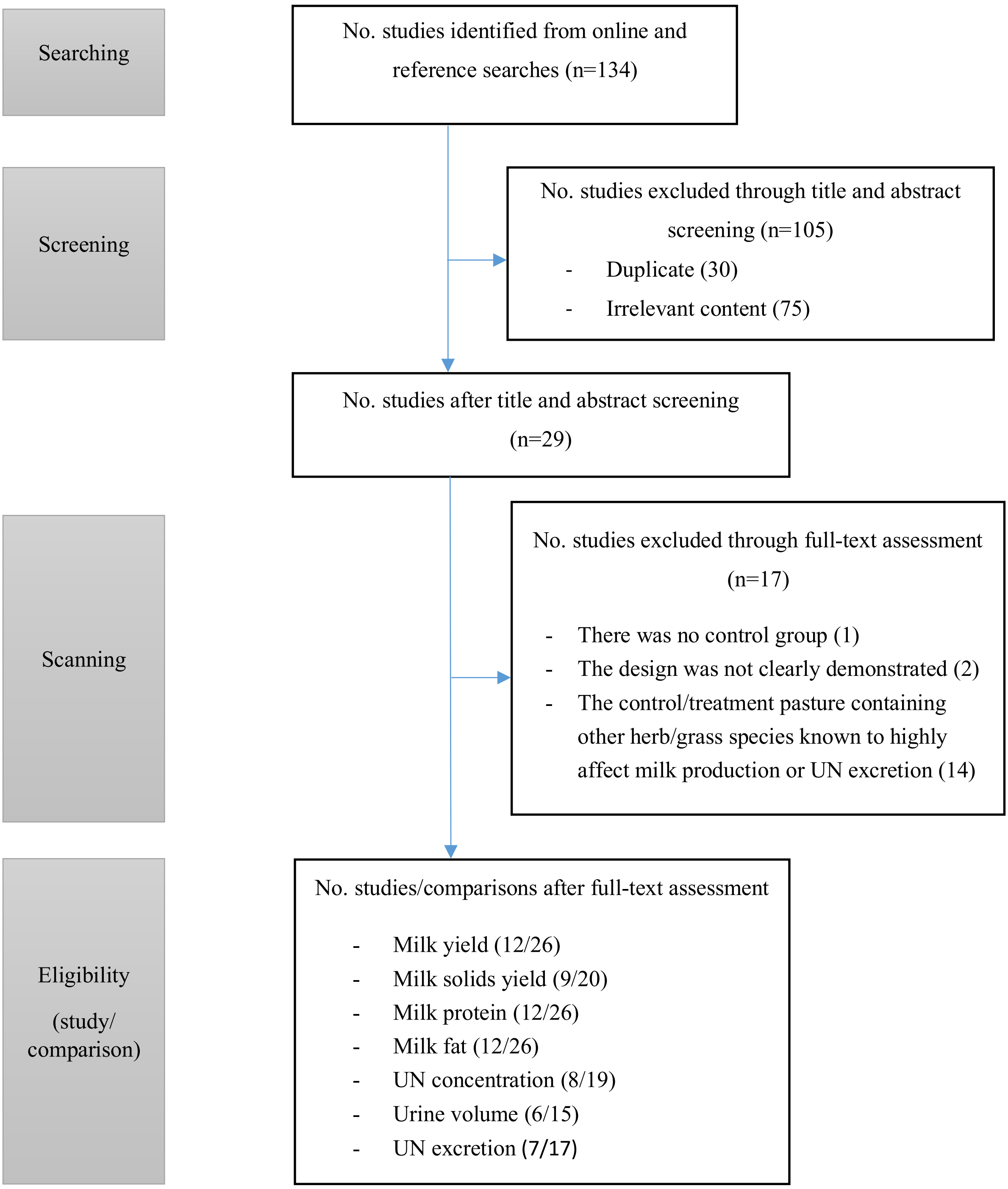


Figure A1.2. The process of literature search and study selection, and the number of studies within each step.

A total of 26 comparisons from 12 studies was included in this meta-analysis. This provided a sample size of 766 dairy cows. Control pastures were mainly ryegrass and white clover, but three contained tall fescue (*Festuca arundinacea* L.) and five included lucerne (*Medicago sativa* L.). Treatment swards were pure plantain or mixed pastures containing plantain with an average content of 434 g/kg DM. The 26 comparisons included 18 in the late, seven in the early lactation period, and one combination of all lactation seasons (Table A1.1). The number of comparisons is enough to conduct a meta-analysis and subgroup analyses for milk yield, milk solids, milk protein concentration, milk protein yield, milk fat concentration, milk fat yield, UN concentration, daily urine volume and UN excretion (Higgins et al., 2021).

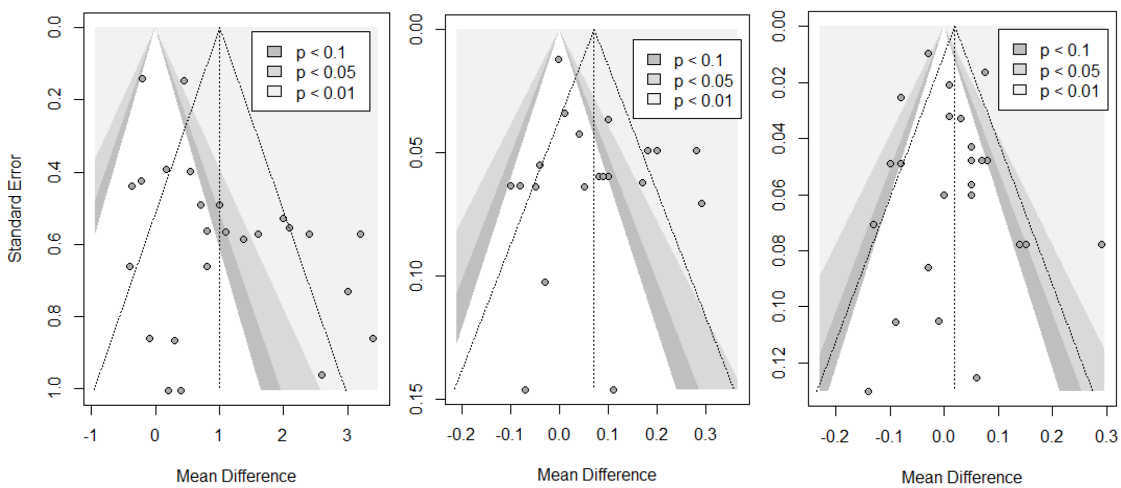
Table A1.1. Summary of studies investigating the effect of pastures containing plantain on milk production and urinary nitrogen excretion of dairy cows.

Studies	**N**	**Control pasture**	**Treatment pasture**	**Farm system**	**Lactation stage**	**Plantain content (g/kg DM)**
Box et al., 2017a	24	RGWC	RGWC-PL	Irrigated	late	499
Box et al., 2017b	24	RGWC	PL	Irrigated	late	896
Box et al., 2017c	24	RGWC	RGWC-PL	Irrigated	early	334
Box et al., 2017d	24	RGWC	PL	Irrigated	early	678
Dodd et al., 2018a	30	TF-LC	TF-LC-PL	Rain-fed	late	510
Dodd et al., 2018b	30	TF-LC	TF-LC-PL	Rain-fed	late	390
Dodd et al., 2018c	30	RG-LC	RG-LC-PL	Rain-fed	early	510
Dodd et al., 2018d	30	RG-LC	RG-LC-PL	Rain-fed	early	370
Mangwe et al., 2018	36	RGWC	WC-PL	NA	late	510
Mangwe et al., 2019	36	RGWC	WC-PL	Irrigated	late	780
Marshall et all., 2020a	48	RGWC	RGWC-PL	NA	early	210
Marshall et all., 2020b	48	RGWC	RGWC-PL	NA	late	210
Marshall et al., 2021	16	RG	PL	NA	late	900
Minnee et al., 2017a	15	RG	RG-PL	NA	late	196
Minnee et al., 2017b	15	RG	RG-PL	NA	late	392
Pembleton et al., 2016a	24	RG	RGWC-PL	NA	early	140
Pembleton et al., 2016b	24	RG	RGWC-PL	NA	late	360

Pembleton et al., 2016c	24	RG	RGWC-PL	NA	early	430
Waghorn et al., 2019	10	TF-LC	TF-LC-PL	Rain-fed	late	179
Minnee et al.,2020a	8	RG	RG-PL	NA	late	170
Minnee et al.,2020b	8	RG	RG-PL	NA	late	330
Minnee et al.,2020c	8	RG	RG-PL	NA	late	450
Nkomboni et al., 2021a	24	RGWC	RGWC-PL	Irrigated	late	210
Nkomboni et al., 2021b	24	RGWC	RGWC-PL	Irrigated	late	390
Nkomboni et al., 2021c	24	RGWC	RGWC-PL	Irrigated	late	680
Al-Marashdeh et al., 2021	158	RGWC	RGWC-PL	Irrigated	All seasons	765

Note: N = number of cows; RGWC = perennial ryegrass-white clover; PL = plantain; TF = tall fescue, LC = lucerne, NA = data is not available or not applicable.

Eggers' test for the funnel plot asymmetry found no significant publication bias for any comparisons with P = 0.072, 0.920, 0.226, 0.803, 0.796, 0.707, 0.743, 0.146, 0.262 for milk yield, milk solids yield, milk protein concentration, milk protein yield, milk fat concentration, milk fat yield, UN concentration, daily urine volume and UN excretion, respectively (Figure A1.3).



(d) (e) (f)

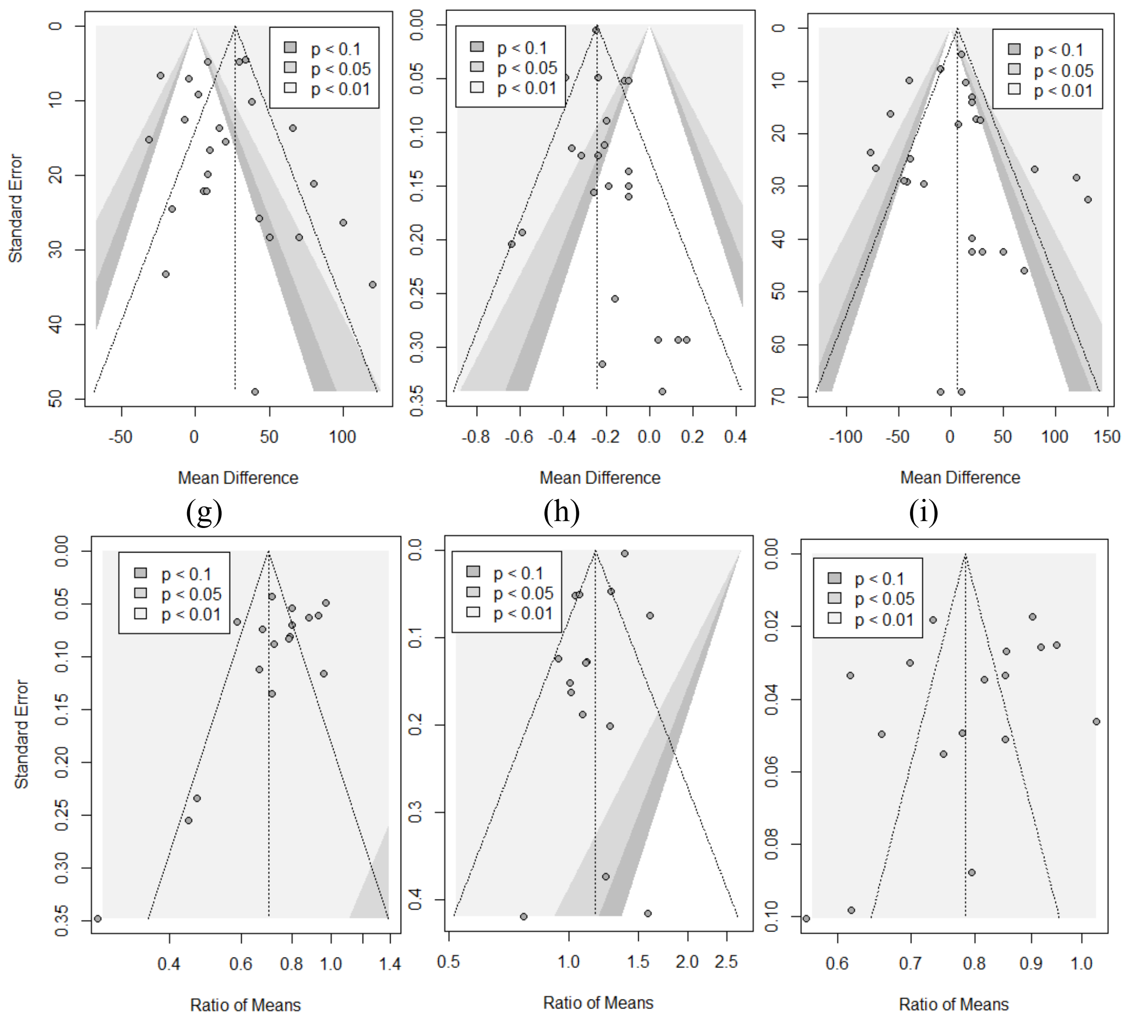


Figure A1.3. Funnel plots; (a) milk yield, (b) milk solids yield, (c) milk protein concentration, (d) milk protein yield, (e) milk fat concentration, (f) milk fat yield, (g) nitrogen concentration in urine, (h) daily urine volume, (i) urinary nitrogen excretion.

2.3. Data extraction

Relevant data were extracted from all selected studies into a database in Microsoft Excel. The database included study characteristics, research design, outcome comparison of milk production, UN excretion, and herbage nutritive values. Outcome data for each comparison were presented with a mean value and a standard deviation.

New variables were created from the extracted data for use as analysed outcomes or independent factors in sub-group analyses. The lactation stage was grouped by days in milk, in which early and late lactations were denoted as 0 to 150 and ≥ 151 days in milk, respectively. If the data were not reported in studies, ME was converted from the digestibility of OM in DM (DOMD), using the equation ME (MJ/kg DM) = DOMD (g/kg DM) × 0.0194 - 2.577 (Freer et al., 2007). Crude protein was obtained from N concentration by the equation CP (g/kg DM) = N (g/kg DM) × 6.25 (Jiang et al., 2014). The quantity of UN excretion was estimated from milk urea nitrogen (MUN), using the equation $UN_{excretion}$ (g/cow/day) = 15.1 × MUN (mg/dL) + 27.8 (Kohn et al., 2002).

Continuous variables of outcomes were extracted with the values of mean and standard deviation (SD). If SD was not reported in studies, it was obtained from standard error of the mean (SEM), standard error of difference (SED), or P-value. Specifically, SD was converted from SEM according to the formula $SD = SEM \times \sqrt{N}$, in which N is the sample size; and from SED using the formula $SD = \frac{SED}{\sqrt{\frac{1}{N_c} + \frac{1}{N_e}}}$, where N_c and N_e refer to the number of cows in the control and treatment groups, respectively. For studies reporting P-value between groups, SD was assumed to be the same for all groups, and it was calculated using mean difference (MD) between two groups and the P-value. In this calculation, the t-value was obtained from the P-value according to the function *[TINV(probability, degree of freedom)]* in Microsoft Excel, where TINV is the t-value (two-tailed), the probability is P-value, and degree of freedom equalled “N-1”. The SEM was then calculated via the equation $SEM = MD/t$ for converting to SD by the above formula. In studies that reported P < 0.01 or < 0.05, P = 0.01 or 0.05 were used for the calculation (Higgins et al., 2021).

2.4. Data analysis

The meta-analysis was conducted using Rstudio, as described by Harrer et al. (2021). A random-effect model was applied for the meta-analysis, using the Meta and Metafor packages (R Core Team, 2021). The population was experimental lactation cows, and the intervention and comparator were pastures either containing plantain or not. The primary outcomes were assessed, including milk yield, milk solids, milk protein (concentration and yield), milk fat (concentration and yield), N concentration in urine, daily urine volume, and total UN excretion. In addition, nutritive values, including DM intake (DMI), CP, ME, ADF, NDF, and water-soluble carbohydrate (WSC) were evaluated for support for the effect of treatment pasture on the main outcomes.

In the meta-analysis, weighted mean differences (WMD) were estimated from the MD or ratio of the mean (ROM) and the weight of individual studies. The MD ($MD = M_{treatment} - M_{control}$) was used for most of the comparations, except UN concentration, daily urine volume and UN excretion that were presented by ROM (ROM = $M_{treatment}/M_{control}$). Relative change (RC) for UN concentration and UN excretion was achieved from ROM via RC (%) = (1 - ROM) × 100. A significant difference was declared when the P-value was less than 0.05, or the 95% CI range did not include a zero value.

Sub-group analysis was conducted to identify the effect of different lactation stages (early: 0–150 DIM and late: ≥ 151 DIM). Moreover, meta-regression was implemented to investigate the impact of the content of plantain in the diet on the outcomes. Regression models were used to estimate the weighted effect size of the relationship when statistical effects were identified. The model was formed by the equation: $\theta_k = \theta + \beta x_k + \epsilon_k + \zeta_k$, where θ_k refers to the effect size of the study k, bx_k is the coefficient of the predictor, ϵ_k and ζ_k denote sampling errors and an overarching distribution of effect size (Higgins et al., 2021).

Heterogeneity among studies was identified for meta-analysis by a chi-squared (χ^2) test with the significance level at $P \leq 0.01$. The I^2 among studies refers to low heterogeneity at 25%, moderate heterogeneity at 50%, and substantial heterogeneity at 75% (Harrer et al., 2021). Also, publication bias within studies was evaluated by funnel plots to identify if low effect size studies were missed in the meta-analysis. The Eggers's test was regarded as significant at $P \leq 0.01$ (Higgins et al., 2021).

3. Results

3.1. Increased milk production

The mean differences of milk yield and composition for lactating cows grazing treatment pastures compared with control pastures are presented in Table A1.2. Overall, milk yield (WMD = 1.02 kg/cow/day, 95% CI = 0.55 to 1.46), milk solids yield (WMD = 0.07 kg/cow/day, 95% CI = 0.02 to 0.12), and milk protein yield (WMD = 23.4 g/cow/day, 95% CI = 11.3 to 35.5) were significantly increased by grazing pastures containing plantain. In contrast, feeding treatment pastures decreased fat concentration in milk (WMD = -0.24 g/100 g milk, 95% CI = -0.31 to -0.17), while milk fat yield and milk protein concentration were maintained. Heterogeneity within studies was substantial for milk fat concentration (I^2 = 57%), milk solids yield (I^2 = 68%), milk protein concentration (I^2 = 71%), milk protein yield (I^2 = 68%), and high for milk yield (I^2 = 79%) and milk fat yield (I^2 = 79%).

Table A1.2. Lactation stage, number of comparisons (N), weighted mean difference (WMD), 95% confidence interval (CI), within studies heterogeneity (I^2), P-value of meta-analysis and subgroup analyses.

Variable	Lactation stage	N	WMD	95% CI	I^2 (%)	P
Milk production						
Milk yield	Overall	26	1.02	0.55 to 1.46	79	<0.001
(kg/cow/day)	Early lactation	7	0.36	-0.33 to 1.05	50	0.407
	Late lactation	18	1.38	0.83 to 1.94	55	<0.001
Milk solids	Overall	20	0.07	0.02 to 0.12	68	0.012
(kg/cow/day)	Early lactation	5	0.02	-0.09 to 0.05	0.0	0.545
	Late lactation	14	0.11	0.05 to 0.17	56	0.002
Milk protein	Overall	26	0.02	-0.02 to 0.06	71	0.274
(g/100 g milk)	Early lactation	7	-0.01	-0.06 to 0.04	57	0.575
	Late lactation	18	0.03	-0.02 to 0.08	58	0.162
Milk protein	Overall	26	23.4	11.3 to 35.5	68	<0.001
(g/cow/day)	Early lactation	7	12.2	-22.7 to 47.0	70	0.410
	Late lactation	18	31.1	14.7 to 47.6	60	0.003
Milk fat	Overall	26	-0.24	-0.31 to -0.17	57	<0.001
(g/100 g milk)	Early lactation	7	-0.13	-0.17 to -0.09	0	<0.001
	Late lactation	18	-0.29	-0.38 to -0.19	55	<0.001
Milk fat	Overall	26	6.3	-14.8 to 27.4	79	0.554
(g/cow/day)	Early lactation	7	-21.9	-53.9 to 10.1	70	0.146
	Late lactation	18	15.4	-3.5 to 34.2	35	0.101
UN concentration and excretion						
UN concentration	Overall	19	0.70	0.62 to 0.80	91	<0.001
(ROM)	Early lactation	4	0.72	0.52 to 0.98	40	0.044
	Late lactation	15	0.70	0.59 to 0.82	93	<0.001
Urine volume	Overall	15	1.17	1.07 to 1.29	85	0.003
(ROM)	Early lactation	5	10.6	0.94 to 1.20	0	0.244
	Late lactation	10	1.19	1.05 to 1.36	77	0.010
UN excretion	Overall	16	0.78	0.72 to 0.85	95	<0.001
(ROM)	Early lactation	4	0.73	0.58 to 0.92	88	0.023
	Late lactation	13	0.80	0.72 to 0.89	96	<0.001
Nutritive characteristics						
DM intake	Overall	22	1.03	0.56 to 1.50	74	<0.001
(kg/cow/day)	Early lactation	6	0.34	-0.83 to 1.51	72	0.487
	Late lactation	16	1.32	0.83 to 1.81	46	<0.001
CP (g/kg DM)	Overall	26	-12.8	-20.4 to -5.1	60	0.002
	Early lactation	7	-6.9	-17.6 to 3.8	0	0.164
	Late lactation	18	-16.0	-26.7 to -5.1	0	0.006
ME (MJ/kg DM)	Overall	26	-0.01	-0.12 to 0.09	88	0.754
	Early lactation	7	0.03	-0.15 to 0.22	74	0.662
	Late lactation	18	-0.02	-0.18 to 0.14	86	0.774
ADF (g/kg DM)	Overall	20	-18.7	-29.2 to -8.4	87	0.001

	Early lactation	5	-7.8	-21.8 to 6.3	73	0.200
	Late lactation	14	-23.7	-38.1 to -9.2	88	0.004
NDF (g/kg DM)	Overall	24	-80.4	-101.9 to -58.9	95	<0.001
	Early lactation	7	-61.1	-87.3 to -35.0	57	0.001
	Late lactation	16	-88.0	-120.2 to -47.8	82	<0.001
WSC (g/kg DM)	Overall	17	8.8	-15.0 to 32.7	92	0.442
	Early lactation	3	-39.9	-129.5 to 49.8	88	0.196
	Late lactation	14	19.3	-04.9 to 43.5	92	0.109

Note: ADF = acid detergent fibre; CP = crude protein; DM = dry matter, ME = metabolisable energy; NDF = non-acid detergent fibre, UN = urinary nitrogen concentration; WSC = water-soluble carbohydrate.

Sub-group analysis by lactation stages showed that most milk production parameters were increased in late lactation, but were similar in early lactation. In late lactation, cows grazing pastures containing plantain had higher milk yield (WMD = 1.4 kg/cow/day, 95% CI = 0.8 to 1.9), milk solids yield (WMD = 0.11, 95% CI = 0.05 to 0.17), and milk protein yield (WMD = 31.1 g/cow/day, 95% CI = 14.7 to 47.6). There were no differences in milk yield, milk solids, protein concentration, and protein yield in early lactation ($P > 0.05$). Milk fat concentration of cows grazing treatment pastures was lower than grazing control pastures in both early lactation (WMD = -0.13 g/100 g milk, 95% CI = -0.17 to -0.09) and late lactation (WMD = -0.29 g/100 g milk, 95% CI = -0.40 to -0.17). The variation between studies was low or moderate for all sub-group comparisons, except for milk protein yield and milk fat yield in early lactation, where it was substantial with I^2 = 70%. Meta-regression analysis conducted to determine the effect of plantain content on milk production, showed no significant correlation with milk yield, milk solids yield, milk protein concentration, milk protein yield, milk fat concentration or milk fat yield.

3.2. Reduced UN concentration and excretion

The meta-analysis showed a significant reduction in UN concentration and excretion for cows grazing treatment pastures, compared to control pastures (Table A1.2). There was an overall reduction of 30% in UN concentration (95% CI = 20 to 38) and 22% in UN excretion (95% CI = 15 to 28), and an increase of 17% in daily urine volume (95% CI = 7 to 29). The heterogeneity within studies in these analyses was high for UN concentration (I^2 = 91%), daily urine volume (I^2 = 85%), and UN excretion (I^2 = 95%). Sub-group analysis showed grazing pastures containing plantain reduced UN concentration, and UN excretion in both early and late lactation stages. However, the effect of plantain on increased daily urine volume was observed only in late lactation.

Meta-regression analysis for the effect of the content of plantain in treatment pastures indicated that increased content of plantain was statistically associated with a reduction in UN concentration (F(df1 = 1, df2 = 17) = 33.8, P < 0.001, R^2 = 69%), a decrease in UN excretion F(df1 = 1, df2 = 15) = 5.3, P = 0.036, R^2 = 22%), and an increase in urine volume F(df1 = 1, df2 = 13) = 28.3, P < 0.001, R^2 = 64%) (Figure A1.4). The estimated curves for these changes are below:

(a) Relative change of UN (%): y = –0.0645 × plantain content (g/kg DM) + 0.61

(b) Relative change of daily urine volume (%): y = 0.0659 × plantain content (g/kg DM) - 12.73

(c) Relative change of UN excretion (%): y = -0.0297 × plantain content (g/kg DM) - 5.86

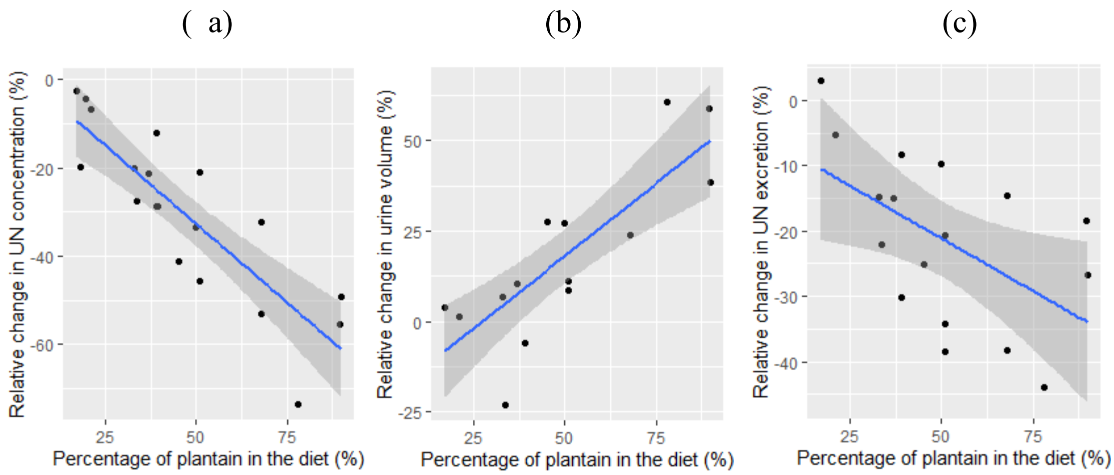


Figure A1.4. The relationship between plantain percentage in the pasture diet and the relative changes in urinary nitrogen (UN) concentration (a), daily urine volume (b), UN excretion (c).

4. Discussion

4.1. Milk yield

Our analysis showed an increase of 1.02 kg/cow/day in milk yield for cows grazing pastures containing an average of 434 g plantain per kg DM versus control pastures over the full lactation across all studies. The change was greater in late lactation than in early lactation, but the increased milk yield was not significantly associated with the content of plantain in the diet. In late lactation, a decline in the nutritive value of RGWC pasture is often a limit to milk production (Kemp et al., 2000). As a drought and heat tolerant herb, plantain can maintain its nutritive value during summer and autumn, especially in dryland systems. In contrast, the good growing conditions in spring and early summer result in the difference in nutritive value between plantain and RGWC not being large enough for a measurable effect of plantain on milk production. The effect of the content of plantain on milk production may be lower than the seasonal effect, and insufficient to provide a linear regression across studies in the present analysis with different experimental conditions. The recorded content of plantain resulting in a significant increase in milk yield ranged from 300 g/kg DM (Minneé et al., 2017) to 900 g/kg DM (Box et al., 2017). However, some studies showed a similar milk yield within this range, either in early lactation (Pembleton et al., 2016) or late lactation (Minnée et al., 2020). This lack of variation in milk yield in response to varying contents of plantain in the pastures is probably because the nutritive value of plantain varied according to its age, development stage and farm condition (Moorhead & Piggot, 2009); therefore, an advantage of pastures containing plantain for milk production was not observed in a number of studies. For example, due to its heat and drought tolerance, plantain can provide a greater quality and quantity of forage feed than ryegrass under rain-fed farm systems and lower rainfall conditions in summer and autumn (Al-Marashdeh et al., 2021). As a result, plantain increases DMI and milk yield in most of the studies conducted under dryland farm systems (Minnée et al., 2020; Waghorn et al., 2019), but maintains DMI and milk yield in studies with irrigated pastures (Al-Marashdeh et al., 2021; Marshall et al., 2020). The differences in experimental conditions among studies could be the reasons causing the high heterogeneity of other outcomes such as milk fat yield and milk protein yield in the present analysis. However, the limited data sources do not allow us to conduct analysis to determine the effect of these factors.

Increased milk production due to plantain is associated with a higher DMI from pastures containing plantain and a change in herbage quality (Minnée et al., 2020; Wilson et al., 2020) However, DMI often varies greatly in grazing studies, so it is difficult to conclude differences within a single study. Previous studies showed different results for DMI from pastures containing plantain compared to traditional pastures, ranging from higher DMI (Box et al., 2017; Bryant et al., 2018) to similar DMI (Minneé et al., 2017; Pembleton et al., 2016). The present analysis of 22 comparisons from nine studies provides clear evidence that offering plantain increased DMI by 1.03 kg/cow/day. Pastures containing plantain have lower concentrations of ADF (-19 g/kg DM) and NDF (-80 g/kg DM), compared to control pastures, requiring less time for rumination and digestion than control pastures (Mangwe et al., 2019). This lower fibre concentration in pastures means that cows can consume and digest more pasture in the same period. In addition, adding plantain into a mixed sward could provide more opportunities for diet selection, assisting cows in having a higher DMI (Pembleton et al., 2016). A higher DMI from pastures containing plantain can result in a higher ME intake by cows, leading to greater milk production. The CP concentration in the plantain diet was slightly lower than in grass-based pastures, by 13 g/kg DM, in the present analysis. However, this is not expected to affect milk production because the mean CP in pastures containing plantain (198 g/kg DM) is adequate for lactating dairy cows (National Research Council, 2001).

4.2. Milk composition

The inclusion of plantain in the diet of dairy cows maintained milk protein concentration and milk fat yield, but increased protein yield, and reduced fat concentration. The increase in protein yield has been reported in previous studies as the main contributor to higher milk solids in cows grazing pastures containing plantain (Box et al., 2017; Wilson et al., 2020). To date, the only potentially negative impact of feeding plantain has been reported to be a depression in milk fat concentration (Dodd et al., 2018; Minnée et al., 2020), although other studies have also reported no effect of plantain on milk fat (Bryant et al., 2018; Nkomboni et al., 2021). In the present analysis, the milk fat concentration of cows grazing treatment pastures was 2.4 g/100 g milk lower than for control pastures. Key drivers for reducing fat concentration in milk may be related to differences in NDF content, the rumen pH and the ruminal passages of plantain and ryegrass. Lower NDF content in plantain could lead to an insufficient supply of acetate for de novo fat production, therefore, depressing milk fat concentration (Palmquist et al., 1993). Moreover, Minneé et al. (2017) indicated a lower pH in the rumen of cows fed plantain than RGWC pasture, which could be caused by a higher content of readily-degradable carbohydrates in plantain forage. This higher content of readily-degradable carbohydrates can restrict lipolysis and inhibit milk fat secretion in dairy cows (Chouinard et al., 1999). In addition, the fast ruminal passage of plantain reduces the exposure of forage lipids to lipolysis and biohydrogenation in the rumen (Dewhurst et al., 2003). If the reduction in milk fat concentration is associated with the change in carbohydrate content and the transformation of specific fatty acids in the rumen, then a supply of additional feed sources with plantain pastures could be an option to balance carbohydrate content. Further studies are required to investigate these effects of plantain and provide solutions to mitigate the decrease in milk fat concentration.

4.3. Nitrogen excretion

This meta-analysis provides strong evidence to support the hypothesis that incorporating plantain in the diet of dairy cows can reduce UN excretion and N-loading onto paddocks. The present study showed a reduction of 30% in urine-N concentration and 22% in total UN excretion, whilst increasing daily urine volume by 17% in cows grazing pastures containing an average of 434 g plantain per kg DM with the range from 140 to 900 g/kg DM. Linear regression curves (a), (b), and (c) allow reasonable estimations for the changes in N concentration, urine volume and UN excretion when the content of plantain in a mixed pasture is identified. These models can explain 69%, 64% and 22% of changes in UN concentration, urine volume and UN excretion, respectively, associated with the content of plantain within the range from 140 to 900 g/kg DM in the diet. More studies are required to improve the accuracy of estimation curves.

A reduction in UN excretion from dairy cows fed plantain has been extensively reported in previous studies (Mangwe et al., 2019; Minnée et al., 2020), and is associated with an increase in urine volume and a decrease in UN concentration in the urine. While the reduction in UN excretion is driven by the content of plantain in the diet, a certain proportion of plantain is required to achieve a significant effect. Most studies agree that diets containing 200 g plantain per kg DM or less do not affect UN concentration (Minneé et al., 2017), urine volume (Bryant et al., 2018), and total UN excretion (Minnée et al., 2020). The content of plantain reported to have a significant effect were 300 g/kg DM for reducing UN concentration, 450 g/kg DM for increasing urine volume (Minnée et al., 2020), and 500 g/kg DM for decreasing UN excretion (Box et al., 2017). The regression models in the present study estimated a reduction of 15, 21 and 27% in UN excretion when 300, 500 and 700 g plantain per kg DM were included in the diet of dairy cows, respectively. However, the effect of plantain on decreasing N losses from farms can be even greater than these figures due to the reduction of N loading at the paddock level. The dilution of N through increased urine volume can spread urine N further throughout the paddock, allowing pastures to uptake more N from the same amount of deposited urine N onto soils (Di & Cameron, 2007). The target content of plantain in swards is suggested to be at least 300 g/kg DM, especially during the critical period between late summer and autumn, to significantly reduce UN excretion onto paddocks. In practice though, at the farm level, it is difficult to maintain plantain content above 30% over an extended period, e.g. beyond three years (Dodd et al., 2019). Further studies are required to investigate the strategies to achieve and maintain this targeted content of plantain in mixed pastures.

A key mechanism for the lower UN concentration in cows eating plantain is the higher proportion of undegradable N (Minnée et al., 2020). The greater undegraded N content allows more N to pass through the rumen to be digested in the small intestine, where more N is partitioned to milk and faeces (Wilson et al., 2020), and less N is excreted into urine (Bryant et al., 2018). Minnée et al. (2020) reported an 11% reduction in N partitioned to urine, and a 14% increase in N partitioned to milk and faeces, when 450 g/kg DM plantain was included in the diet. Moreover, the higher water and mineral contents in plantain forage are potential causes of the increase in daily urine volume. Water content in plantain can be 5% higher than in ryegrass, resulting in a higher water intake in diets with more plantain (Minnée et al., 2020). The concentration of calcium, sodium (Na) and potassium (K) in plantain are higher than in ryegrass (Box et al., 2018; Sanderson et al., 2003). High Na and K contents have been suggested as an explanation for increased urine volume from cows grazing plantain (Bannink et al., 1999; Spek et al., 2012). Mangwe et al. (2019) indicated that cows grazing plantain consumed 5.4 and 1.3 times more Na and K and urinated 1.6 times more urine than cows grazing RGWC pasture. In addition, the reduction in UN excretion might also be related to aucubin in plantain, which is associated with a reduction in ammonia production in the rumen fluid and causes diuresis (O'Connell et al., 2016).

5. Conclusion

The present review quantified the effect of plantain in pastures on increasing milk production and decreasing UN excretion of dairy cows. The analysis showed that grazing pastures containing an average of 43% plantain increased the yield of milk, milk solids, and milk protein, with a greater increase in late lactation. Fat concentration in milk was slightly reduced, but milk fat yield was maintained due to increased overall milk solids. Moreover, feeding plantain pastures reduced UN concentration and total UN excretion, while increasing the urine volume of dairy cows with the responses linear over the content of plantain within the range of 140 to 900 g/kg DM. These changes in N output can result in a lower N loading at paddock level, contributing to decreased N lost from dairy farms to the environment.

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Appendix 2. Herbage yield, botanical composition, nutritive value, the content of minerals and bioactive compounds of pasture treatments.

Variable	Spring				Early summer				Late summer				Spring				SEM
	RGWC	PLL	PLM	PLH	RGWC	PLL	PLM	PLH	RGWC	PLL	PLM	PLH	RGWC	PLL	PLM	PLH	
Pasture production																	
Pre-grazing mass (kg DM/ha)	3,812	3,664	3,451	3,724	4,021	3,944	3,818	4,007	3,268	3,362	3,136	3,344	3,273	3,316	3,122	3,055	419
Post graze mass (kg DM/ha)	1,855	1,779	1,549	1,654	2,125	2,058	1,948	2,142	2,089	1,995	1,799	1,941	2,029	1,941	1,750	1,630	450
Botanical composition																	
Plantain leaves (%)	3.7	22.6	33.1	38.5	2.1	18.6	28.0	30.3	3.9	23.0	33.8	36.7	3.8	31.0	43.1	45.1	3.0
Plantain stem (%)	1.2	6.1	8.2	8.9	1.2	12.8	17.9	17.6	0.5	8.4	8.2	9.9	1.7	4.3	3.8	4.5	2.1
Perennial ryegrass (%)	78.5	57.5	46.0	41.3	69.2	43.4	28.7	28.5	44.7	22.8	17.5	13.6	54.1	31.5	22.6	20.7	7.2
White clover (%)	8.1	7.7	8.8	7.7	17.7	16.6	19.4	17.6	25.3	20.1	20.6	19.7	15.8	13.5	14.5	12.7	5.8
Dead material (%)	6.8	5.0	2.9	3.1	8.2	6.4	5.0	4.5	24.9	24.4	18.9	18.4	24.2	19.0	15.8	16.5	1.4
Nutritive value																	
DM (%)	16.2	25.9	22.6	17.1	20.3	34.7	31.9	21.0	17.8	30.0	25.0	18.3	16.2	26.4	22.9	17.9	2.10
OMD (g/kg DM)	697	709	718	781	698	672	666	768	701	679	695	776	704	716	731	786	1.40
CP (g/kg DM)	183	188	188	183	186	198	203	197	180	178	183	185	194	190	201	203	2.27
ADF (g/kg DM)	251	248	235	231	284	275	275	279	274	276	256	258	267	261	249	251	0.65
NDF (g/kg DM)	429	403	371	362	498	476	435	447	470	450	409	412	465	431	397	400	1.01
Soluble sugars (g/kg DM)	88	82	90	95	70	63	56	49	63	57	62	59	65	69	73	67	1.13
NSC (g/kg DM)	243	261	291	279	180	192	225	221	219	238	265	262	200	237	251	251	1.86
ME (MJ/kg DM)	111	112	113	144	99	100	100	99	96	97	101	100	95	98	102	101	0.94
Mineral content																	
Phosphorus (g/kg DM)	3.90	4.15	4.11	4.22	3.61	4.08	4.38	4.32	3.35	3.71	3.96	3.96	2.95	3.06	3.30	3.22	0.02
Sulfur (g/kg DM)	2.62	2.84	2.90	2.96	2.91	3.06	3.32	3.21	2.88	3.21	3.56	3.52	2.95	3.13	3.42	3.47	0.01
Calcium (g/kg DM)	8.5	11.1	13.2	13.4	7.2	10.4	13.8	13.5	9.2	13.4	15.8	15.9	8.6	12.9	15.6	15.6	0.07
Sodium (g/kg DM)	3.70	4.48	5.02	4.93	2.73	3.83	4.75	4.56	4.26	6.10	7.05	6.81	3.81	5.70	6.88	6.53	0.05
Chlorine (g/kg DM)	13.8	16.9	19.1	17.7	11.1	12.5	12.1	12.9	12.8	15.8	16.8	16.0	13.4	17.6	20.9	21.4	0.19
Bioactive compound																	
Aucubin (mg/g DM)	0.33	1.18	1.76	2.70	0.53	2.57	3.88	4.71	1.01	4.10	6.04	7.24	0.54	4.66	7.35	7.78	0.49
Acteoside (mg/g DM)	0.17	2.05	3.14	4.61	0.79	3.65	6.01	7.50	0.88	6.39	9.75	10.83	0.38	4.34	8.27	8.34	0.44

Note: ADF = acid detergent fibre, CP = crude protein, DM = dry matter, ME = metabolisable energy, NDF = neutral detergent fibre, NSC = non-structural carbohydrates, OMD = organic matter digestibility, RGWC = perennial ryegrass-white clover, PLL = RGWC + low plantain rate, PLM = RGWC + medium plantain rate, PLH = RGWC + high plantain rate, SEM = standard of the means.

Appendix 3. Equation to estimate urine patch area

Urine patch area (m^2/cow/day) = daily urination volume (m^3) ÷ urine column (m) × number of urination per day (Romera et al., 2012),

where the urine column is assumed to be 10 mm (Selbie et al., 2015).

Appendix 4. Equation to estimate nitrogen (N) load at urine patch.

N load at urine patches (kg/ha) = urinary N (UN) excretion (kg) ÷ urine patch area (ha) (Romera et al., 2012),

where UN excretion (kg) = urine volume (L) × UN concentration (kg/L).

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Appendix 5. Herbage characteristics, milk production and N excretion of dairy cows in pasture treatments.

Variable	Autumn				Early summer				Late summer				SEM
	RGWC	PLL	PLM	PLH	RGWC	PLL	PLM	PLH	RGWC	PLL	PLM	PLH	
Herbage diet													
Herbage intake (kg DM/cow/day)	14.4	15.9	16.4	17.4	16.3	16.1	15.7	15.8	16.1	18.0	17.9	19.1	**2.22**
Plantain in the diet (%)	3.8	31.0	43.1	45.1	2.1	18.6	28.0	30.3	3.9	23.0	33.76	36.74	**2.24**
DM (g/kgFW)	319	250	229	226	203	178	162	162	347	300	264	259	**7.04**
CP (g/kg DM)	194	190	199	199	186	199	199	197	180	178	183	185	**5.68**
ADF (g/kg DM)	267	253	253	247	284	275	275	275	274	271	256	254	**5.64**
NDF (g/kg DM)	465	431	404	395	498	476	435	447	470	450	409	412	**10.9**
NSC (g/kg DM)	200	237	251	254	180	192	225	221	219	238	265	262	**9.5**
ME (g/kg DM)	9.5	9.8	10.2	10.1	9.9	10.0	10.0	10.0	9.6	9.7	10.1	10.1	**0.15**
Aucubin (g/kg DM)	0.54	4.66	7.35	7.78	0.53	2.57	3.88	4.71	1.01	4.10	6.04	7.24	**0.47**
Milk production													
Milk yield (L/cow/day)	22.2	22.4	22.5	22.6	25.5	26.1	26.5	26.5	25.4	24.7	24.8	25.6	**0.42**
Milk solids (kg/cow/day)	2.89	2.89	2.93	2.90	3.33	3.55	3.53	3.58	3.30	3.24	3.15	3.37	**0.07**
Protein (kg/cow/day)	0.73	0.75	0.74	0.73	0.79	0.84	0.85	0.87	0.83	0.83	0.82	0.86	**0.02**
Fat (kg/cow/day)	0.72	0.76	0.73	0.78	1.33	1.40	1.42	1.34	1.16	0.97	1.04	1.14	**0.04**
Lactose (kg/cow/day)	1.19	1.22	1.21	1.22	1.30	1.36	1.38	1.37	1.42	1.33	1.38	1.43	**0.04**
MUN (mg/dL)	11.2	9.9	8.6	9.2	10.7	9.9	9.8	9.8	10.3	9.1	8.7	9.0	**0.18**
Urine production and N excretion													
Urine volume (L/day)	29.7	36.4	42.4	41.6	25.2	31.3	31.7	33.9	30.9	37.8	44.0	45.5	**1.53**
Urine N concentration (%)	0.65	0.57	0.44	0.43	0.69	0.57	0.58	0.57	0.53	0.44	0.35	0.36	0.02
Faecak N concentration (%)	2.42	2.51	2.54	2.62	2.40	2.55	2.55	2.65	2.46	2.54	2.56	2.64	0.03
Urine N excretion (g/day)	193	192	167	162	174	174	173	177	168	158	147	159	**5.84**
Milk N (g/day)	96	102	102	96	126	134	137	136	124	123	121	125	**2.37**
Faecal N excretion (g/day)	116	118	118	128	118	121	123	125	129	134	132	141	**1.39**

Note: ADF = acid detergent fibre, CP = crude protein, DM = dry matter, ME = metabolisable energy, MUN = milk urea nitrogen, N = nitrogen, NDF = neutral detergent fibre, NSC = non-structural carbohydrates, RGWC = perennial ryegrass-white clover, PLL = RGWC + low plantain rate, PLM = RGWC + medium plantain rate, PLH = RGWC + high plantain rate, SEM = standard of the means.

Appendix 6. Calibration model for the concentration of milk solids, fat, protein, and milk urea nitrogen, analysed by MilkoScanTM FT1.

Variable	Intercept	FOSS	SE_{FOSS}	P_{FOSS}	R^2
Milk fat	0.11	0.87	0.014	<0.001	0.91
Milk urea nitrogen	-0.28	0.69	0.028	<0.001	0.79
Milk solids	1.79	0.87	0.009	<0.001	0.90
Milk protein	-0.13	0.97	0.007	<0.001	0.95

Note: FOSS = data analysed by MilkoScan™ FT1, SE = standard error, R^2 = coefficient of determination.

Appendix 7. Herbage production, nitrogen (N) losses, and N components in the pastoral system in 2020 and 2021.

Variables	2020					2021				
	RGWC	PLL	PLM	PLH	SEM	RGWC	PLL	PLM	PLH	SEM
Pastures										
Herbage yield (kg DM/ha)	14,281	14,225	14,761	14,979	434	12,676	13,439	13,696	12,348	434
Stock density (cow/ha)	2.7	2.7	2.7	2.7	-	2.5	2.5	2.5	1.9*	-
Plantain composition (g/kg DM)	6.2	40	54	57	1.8	0.3	21	30	47	1.8
White clover (g/kg DM)	15	12	13	12	1.8	17	19	19	30	1.8
Aucubin (g/kg DM)	0.8	4.2	6.2	7	0.42	0.2	2.3	4.4	4.4	0.42
N losses										
Drainage volume (mm/year)	131	116	134	128	14	284	267	286	305	14
Nitrate concentration (ppm)	8.8	4.6	3.2	3.8	0.59	8.7	7.5	5.1	5.3	0.59
Mean total N concentration (ppm)	10.3	7.4	6.9	6.7	0.62	9.9	8.7	6.4	6.9	0.62
Nitrate leaching (kg/ha/year)	11.5	5.1	3.9	4.2	1.39	24.9	19.7	13.5	16.9	1.39
Total N leaching (kg/ha/year)	13.7	8.3	9.3	7.4	1.9	41.5	30.7	26.5	29.9	1.9
N input and output										
Urinary N (kg N/ha/year)	160	167	148	163	23	226	207	192	159	20
Faecal N (kg N/ha/year)	144	144	141	157	7	136	144	138	110	7
BNF (kg N/ha/year)	59	50	57	49	8.1	61	71	73	103	8.1
Herbage N uptake (kg N/ha/year)	654	568	458	498	60	581	472	424	391	52
Urination and urine patches										
Urine patch area (% paddock)	22	27	30	29	1.7	34	38	38	34	1.6
N load on patches (kg N/ha/year)	629	558	444	475	22	568	472	427	390	21

Note: BNF = biological nitrogen fixation, DM = dry matter, N = nitrogen, NO_3^- = nitrate, RGWC = perennial ryegrass-white clover, PLL = RGWC + low plantain rate, PLM = RGWC + medium plantain rate, PLH = RGWC + high plantain rate, SEM = standard of the means.

Appendix 8. Statement of contribution forms (DRC-16)

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Nguyen Truong Thi
Name/title of Primary Supervisor:	Peter Kemp
In which chapter is the manuscript /published work:	Chapter 2

Please select one of the following three options:

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Nguyen, T.T.; Navarrete, S.; Horne, D.J.; Donaghy, D.J.; Kemp, P.D. Forage plantain (Plantago lanceolata L.): Meta-analysis quantifying the decrease in nitrogen excretion, the increase in milk production, and the changes in milk composition of dairy cows grazing pastures containing plantain. Anim. Feed Sci. Technol. 2022, 285, 115244. https://doi.org/10.1016/j.anifeedsci.2022.115244.

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Candidate's Signature:	Nguyen Truong Thi — Digitally signed by Nguyen Truong Thi Date: 2023.02.01 12:33:02 +13'00'
Date:	25-Feb-2023
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We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

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In which chapter is the manuscript /published work:	Chapter 3

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  Nguyen, T. T., Navarrete, S., Horne, D., Donaghy, D., Bryant, R. H., & Kemp, P. (2023). Dairy Cows Grazing Plantain-Based Pastures Have Increased Urine Patches and Reduced Urine N Concentration That Potentially Decreases N Leaching from a Pastoral System. Animals, 13(3), 528. https://www.mdpi.com/2076-2615/13/3/528

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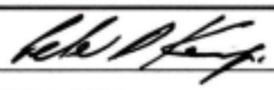
Primary Supervisor's Signature:	
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