Can plantain maintain milk production of dairy cows whilst reducing urinary N losses?

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy in Agricultural Science

at

Lincoln University

by

Lisa Anne Box

Lincoln University

2017

Abstract of a thesis submitted in partial fulfilment of the

requirements for the Degree of Doctor of Philosophy.

Can plantain maintain milk production of dairy cows whilst reducing urinary N losses?

by

Lisa Anne Box

Nitrogen (N) from urine patches is a major contributor to N leaching, due to the high loading rate of N in urine patches compared with the capacity of many plant species to take up N. This study aimed to determine milk production and urinary N excretion from dairy cows grazing a single alternative forage; plantain; to compare the effect of time of day and N fertiliser inputs on the chemical composition of alternative forages which may prove useful in reducing the supply of N to the grazing animal and; to investigate management strategies around time of day allocation which allow for at least 50% of the animals diet to be plantain. Five experiments were conducted in Canterbury, New Zealand which compared forages in grazing, small plot and *in sacco* studies.

In the first experiment, the effects of 50% or 100% of a herbage diet of plantain on milk production and urinary N concentration were measured in two experiments for late (autumn 2015) and early (spring 2015) lactation spring calving cows. Three groups of 12 mixed age Friesian × Jersey dairy cows were offered a perennial ryegrass-white clover pasture, pure plantain or 50% perennial ryegrass-white clover and 50% pure plantain by ground area (50–50 pasture–plantain). Cows were blocked according to milksolids, age, days in milk and liveweight. The experiment was conducted for 10 days with a 3 day transition period and 7 day experimental period. Urine N concentration was lower in the late and early lactation experiments ($P < 0.001$) for plantain (2.4 and 2.2 g N/L respectively) and 50–50 pasture–plantain (3.6 and 3.4 g N/L respectively) than pasture (5.4 and 4.7 g N/L respectively). There was an increase in urine output in late lactation from cows on plantain (73.8 L/cow/day) and 50-50 pasture-plantain (59.1 L/cow/day) compared with those on perennial ryegrass-white clover (46.5 L/cow/day). In early lactation urine volume was similar among treatments averaging 43.8 L/cow/day. The increase in urine volume in late lactation did not offset the large reduction in urinary N concentration, resulting in a 30% reduction in urinary N output from cows grazing 100% plantain (177 and 119 g N/cow/day in late and early lactation respectively) and 15-30% reduction for those on 50% plantain (213 and 114 g N/cow/day in late and early lactation respectively) compared with those grazing perennial ryegrass-white clover (251 and 205 g N/cow/day in late and early lactation respectively). An increase in urine frequency was observed in both experiments for cows on 50% or 100% plantain compared with those on pasture which may result in a better spread of urine when cows are fed plantain. Milk solids produced per cow in late lactation were greatest for cows grazing plantain (1.67 kg/day) compared to pasture (1.50 kg/day) with 50–50 pasture–plantain intermediate (1.60 kg/day). Milk solids production was similar among treatments in early lactation averaging 2.41 kg/cow/day. This experiment suggests plantain may offer environmental benefits to dairy systems by reducing the N concentration of urine deposited on the soil from grazing cows without impeding production.

In the second experimental chapter, a plot experiment was carried out to examine the effects of nitrogen inputs (low (180 kg N/ha/year for grasses and herbs and 156 kg N/ha/year for legumes) and high (450 kg N/ha/year for grasses and herbs and 389 kg N/ha/year for legumes)) and harvesting time (0700 h (AM) vs 1600 h (PM)) on the chemical composition of six forages (perennial and Italian ryegrass, plantain, chicory and red and white clover). The experiment was managed as a cut and carry system with forages harvested when they reached grazing height. Nitrogen fertiliser was applied following each harvest in the form of calcium ammonium nitrate (CAN 27-0-0-0). The effect of harvest time was greater ($P<0.001$) than the effects of N fertiliser on chemical composition for all forages. Perennial and Italian ryegrass showed the largest increase in WSC from morning to afternoon, at the expense of NDF and to a lesser extent CP. This suggests afternoon allocation of perennial or Italian ryegrass may be beneficial to improve the nutritive value of pasture on offer and allocation timing is less important for white clover, chicory and plantain. Chicory and plantain, maintained a greater CP concentration at low N fertiliser inputs (averaging 15.3 g CP/kg DM) than the grasses (averaging 12.6 g CP/kg DM) in summer and autumn. Where N fertiliser use is limited herbs may provide a suitable alternative to meet an animal CP requirements. The higher CP of chicory and plantain may lead to greater N intake per unit DM consumed for herbs compared with perennial and Italian ryegrass. This indicates that factors other than N intake may be driving the lower N excretion seen for cows grazing plantain in Chapter 3.

The third experiment, was an *in sacco* experiment which evaluated the effects of harvesting perennial ryegrass-white clover or plantain in the morning (0700 h) or afternoon (1600 h) on the degradation characteristics in the rumen of dairy cows. The experiment was a 2 × 2 factorial design. There were two pasture treatments of either plantain or perennial ryegrass-white clover which were harvested at two times of the day; 0700 hrs or 1600 hours. This created four treatments. Four rumen cannulated multiparous Jersey × Friesian lactating dairy cows were used. Each cow received one treatment, repeated four times until all cows received each treatment. Forages harvested in the afternoon had a greater ($P<0.001$) concentration of WSC (20.2 and 13.2% DM for plantain and perennial ryegrass-white clover respectively) compared with those harvested in the morning (14.9 and 8.36% DM for plantain and perennial ryegrass-white clover respectively). This came at the expense of NDF and to a lesser extent CP. However, the difference in NDF between forage types (averaging 26.1% for plantain and 40.9% for perennial ryegrass-white clover) was greater ($P=0.012$) than effect of harvest time. Overall the effect of altering the forage type was greater than the diurnal effect on rumen degradation characteristics. Plantain had faster ($P<0.001$) DM degradation rates than perennial ryegrass-white clover which resulted in faster ($P=0.004$) N degradation rates. Faster DM degradation rates are correlated with improved voluntary feed intakes. As a result the use of plantain as pasture for dairy systems may be useful to increase total voluntary intake.

The fourth experiment compared milk production and urinary N from late lactation cows using allocation methods which provided a 50% perennial ryegrass-white clover and 50% plantain diet. Using results from the plot and *in sacco* experiments, three replicated groups of four late lactation, Holstein Friesian x Jersey dairy cows were blocked according to milksolids, age, days in milk and liveweight and assigned to one of two treatments of 50% plantain and 50% perennial ryegrass either as (1) spatially separated monocultures in the same paddock (spatial separation), or (2) plantain allocated following morning milking and perennial ryegrass allocated following afternoon milking (temporal separation). The experiment was conducted for 14 days comprised of a 7 day adaptation period and 7 day experimental period. Milksolids production was greater ($P=0.001$) for cows grazing spatial (1.53 kg MS/cow/d) than temporal (1.37 kg MS/cow/d) separation treatments. Apparent DM intake (15.0 kg DM/cow) and urine N concentration (2.6 %) was similar ($P>0.05$) between treatments. Under the conditions of the experiment, allocating plantain temporally resulted in lower milk production. When offering a diet of 50% plantain - 50% perennial ryegrass, this experiment suggested allocation for greatest milk production is best achieved through spatial separation.

A feature of the results from the grazing experiment of this study was the reduction in urinary N concentration when cows grazed plantain, despite similar N intake to cows grazing perennial ryegrass-white clover. Other research suggests this may be due to plant secondary metabolites aucubin, acteoside and catalpol. A final experiment aimed to characterise the concentration of secondary metabolites in plantain herbage. This was a two year experiment using plantain from the plot experiment. The experiment was a randomised block design with three replicated treatments. Treatments were two N fertiliser rates (180 kg N/ha/year and 450 kg N/ha/year) and two harvest times (0700 h and 1600 h). Samples were taken seasonally when plantain had reached grazing height. Concentrations of the metabolites were varied. Acteoside consistently had the greatest concentration (ranging from 36.0-1.77 mg/g DM), aucubin intermediate (range 5.85-0.566 mg/g DM) and catalpol lowest (<0.390 mg/g DM). The concentration of metabolites was not be altered by N fertiliser and typically did not show diurnal fluctuations. There was a strong seasonal effect on the metabolites with peaks in concentration occurring in spring. This experiment suggests N fertiliser and time of day harvesting cannot be used as strategies to increase the concentration of secondary metabolites in plantain.

This study confirmed that plantain has similar feed value and milk production potential to perennial ryegrass-white clover when offered as green leafy herbage to dairy cows. However, feeding plantain at 50 or 100% of the diet resulted in significant reduction in both urine N concentration and urine N excretion. As these are key components of determining N loading of urine patches, the study indicates that plantain may present an appealing opportunities to reduce the environmental impact of dairy farming. This was not attributed to a lower CP of plantain but may be related to the secondary metabolites in plantain herbage.

Keywords: crude protein, forage, leaching, *Lolium perenne*, nitrate, perennial ryegrass, *Plantago lanceolata* L., *Trifolium repens*, white clover

Acknowledgements

I wish to extent my thanks to my supervisor, Professor Grant Edwards, for his support, guidance, encouragement and constructive criticism for the past 3 years. To my associate supervisor Dr Racheal Bryant, thank you for your guidance and invaluable assistance throughout my data collection and your dedication to getting the best out of my writing.

I would like to acknowledge the Forages for Reduced Nitrate Leaching programme in partnership with DairyNZ, AgResearch, Plant and Food Research, Lincoln University, Foundation for Arable Research and Landcare Research with principal funding from Ministry of Business Innovation and Employment. Working on such relevant research and having multiple opportunities to present my research back to farmers has been a real privilege.

Thanks to the help of the farm staff and technicians at the Lincoln University Research Dairy Farm for your support in data collection during my grazing and rumen degradation experiments. It can't be easy juggling commercial interests, students and scientists while managing cows, pastures and irrigators. Thank you to Dave Jack and Dan Dash for your assistance in establishing the plantain paddocks and small plots experiment.

I would like to acknowledge Neil Smith for all his time in the development and maintenance of the urine harness.

A big thank you to the Lincoln University animal science post graduate students working alongside me at LURDF. The support we have been able to provide one another, particularly during our experiments, has been amazing and I am grateful for all the times you leant me a hand.

Thanks to Amy for being such a supportive friend and for the many coffee and lunch dates. Keep shining bright.

I am grateful to have multiple families who love and support me. Your encouragement and willingness to hear all about plantain over the past few years has been great. To Mum and Dad, without you I wouldn't be where I am today.

To Brian, your love and support throughout all my endeavours over the last three years (and those prior) has meant more than the world to me. I will be forever grateful to have achieved this goal with your support and I look forward to the opportunities that this creates for us.

Table of Contents

Chapter 7 Seasonal and diurnal change in the secondary metabolite composition of plantain over two growing seasons with high and low N fertiliser inputs......................95

List of Tables

List of Figures

List of Plates

List of Abbreviations

Abbreviation	**Description**	**Unit**
A	Soluble fraction	%
ADF	Acid detergent fibre	% of DM; g/kg DM
ANOVA	Analysis of variance	
B	Degradable insoluble fraction	%
Bo	Boron	% of DM; g/kg DM
C	Fractional disappearance rate	%/hour
Ca	Calcium	% of DM; g/kg DM
Cl	Chloride	% of DM; g/kg DM
Co	Cobalt	% of DM; g/kg DM
CP	Crude protein	% of DM; g/kg DM
Cr	Chromium	% of DM; g/kg DM
Cu	Copper	% of DM; g/kg DM
DCAD	Dietary cation-anion difference	
DCD	Dicyanamide	
DM	Dry matter	kg; %
DMI	Dry matter intake	kg; %
DOMD	Digestible organic matter	% of DM; g/kg DM
ED	Effective degradability	%
ERDP	Effective rumen degradable protein	
FCE	Feed conversion efficiency	
Fe	Iron	% of DM; g/kg DM
HSG	High sugar grasses	
Ital.	Italian	
K	Potassium	% of DM; g/kg DM
LSD	Least significant difference	
LWT	Liveweight	
ME	Metabolisable energy	MJ ME
Mg	Magnesium	% of DM; g/kg DM
Mn	Manganese	% of DM; g/kg DM
Mo	Molybdenum	% of DM; g/kg DM
MUN	Milk urea nitrogen	
N	Nitrogen	% of DM; g/kg DM

N_2O	Nitrous oxide	
Na	Sodium	% of DM; g/kg DM
NDF	Neutral detergent fibre	% of DM; g/kg DM
NH_3	Ammonia	
NIRS	Near infrared reflectance spectroscopy	
NO_{3-}	Nitrate	
NUE	Nitrogen use efficiency	
OM	Organic matter	%
P	Phosphorus	% of DM; g/kg DM
PD	Purine derivative	
Per.	Perennial	
pH	Potential of hydrogen	
RG	Ryegrass	
RPM	Rising plate meter	
S	Sulphur	% of DM; g/kg DM
Se	Selenium	% of DM; g/kg DM
SEM	Standard error of the mean	
WSC	Water soluble carbohydrates	% of DM; g/kg DM
Zn	Zinc	% of DM; g/kg DM

Chapter 1

Introduction

1.1 Introduction

Dairy farming in New Zealand is based predominately on year round outdoor grazing systems which give the advantage of producing milk at a low cost (Holmes *et al.* 2007). The systems rely on the predominat use of a binary mix of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). Whilst this pasture is productive under grazing, easy to manage and persists well (Kemp *et al.* 1999), issue arise around the inefficient use of nitrogen (N) (Di *et al.* 2002a). The pasture is reliant on N ferstiliser inputs which contribute to a high N content. The N concentration of perennial ryegrass-white clover pastures is typically 3-4% which is above the requirement of a grazing dairy at approximately 2.8% (CSIRO 2007). Nitrogen in excess of an animals requirements is excreted predominately in the urine. The small scale of the urine patch results in N being deposited at rates of up to 800 kg N/ha which is exceeds plant uptake capactiy (Di *et al.* 2002a). Nitrogen from the urine is easily converted to nitrate (NO_{3-}) which is loosely bound to soil particles. Nitrate is easily leached through the soil profile and into waterways. Nitrogen in waterways is of environmental concern and has contributed to the decline in water quality throughout New Zealand. Regional Councils throughout New Zealand have been developing regulations that place a limit on the amount of N leaching from agricultural land. A substantial reduction in N leaching from current dairy farm levels may be required. Mitigation strategies which reduce N losses from dairy systems whilst maintaining production and New Zealand's competitive advantage are sought. Such strategies include the use of alternative forages.

Previous research has focused on the use of multi-species forages for maintaining milk production whilst reducing urinary N losses (Woodward *et al.* 2012; Totty *et al.* 2013; Edwards *et al.* 2015; Bryant *et al.* 2017). These experiments compared milk production and urinary N concentration from cows grazing either a perennial ryegrass-white clover pasture or a diverse pasture which always contained additions of herbs; plantain (*Plantago lanceolata* L.) and chicory (*Cichorium intybus*), and often also contained additional grasses (such as Italian ryegrass (*Lolium multiflorum*) and tall fescue (*Festuca arundinacea*)) and additional legumes (including lucerne (*Medicago sativa*)) to perennial ryegrass and white clover. A feature of the results from diverse pasture experiments was a reduction of at least 20% in urinary N concentration with no penalty on milk production. Further modelling using data from the grazing experiments suggested diverse pastures as a suitable strategy for reducing N leaching on New Zealand dairy farms (Beukes *et al.* 2014). A consistent finding of experiments which investigated diverse pastures was that their benefits were due to the attributes of the additional species, rather than increasing the number of species per se. Therefore identifying a single forage species which can provide large reductions in urinary N losses without impeding production could prove the most useful tool to improve the sustainability of dairy systems.

Of the forages included in diverse pastures, the herbs, plantain and chicory, have been identified as key drivers behind reducing urinary N concentration and subsequent risk of N leaching (Vibart *et al.* 2016). The unique chemical composition of plantain (Stewart 1996; Tamura *et al.* 2002; Gardiner *et al.* 2016; Navarrete *et al.* 2016) makes it likely to have caused a greater reduction in urinary N than any of the other forages used in diverse pasture experiments (Woodward *et al.* 2012; Totty *et al.* 2013; Edwards *et al.* 2015; Bryant *et al.* 2017). Plantain has a high mineral content and contains secondary plant metabolites (aucubin, catalpol and acteoside) which may have a diuretic effect. The high mineral content and secondary metabolite content of plantain combined with a lower dry matter (DM) % may contribute to increased urine volumes thereby diluting N in the urine. However, there is currently no data on the effects of plantain as a monoculture on milk production, urine composition and total urine output.

A further strategy to reduce N losses in the urine is by reducing N intake. This has been achieved previously by increasing water soluble carbohydrate (WSC) concentration at the expense of crude protein (CP) (Miller *et al.* 1999; Miller *et al.* 2000; Moorby *et al.* 2006). Perennial ryegrass is known to have a diurnal accumulation of WSC (Miller *et al.* 2001). This increase in WSC comes at the expense of both CP and fibre. An increase in WSC at the expense of CP alters the ratio of WSC to CP which can improve N use efficiency (NUE) and reduce N losses in the urine (Edwards *et al.* 2007). The diurnal change in chemical composition has been well described for perennial ryegrass, however, there is currently little research around the diurnal change in chemical composition of alternative forages. A forage which can increase the concentration of WSC predominately at the expense of CP, has the potential to provide a better synchrony supply of nutrients to the rumen and improve NUE of a feed which could reduce N losses in the urine and subsequently reduce the risk of N leaching.

1.2 Aim and research objectives

The aim of this research was to determine milk production and urinary N excretion from dairy cows grazing plantain compared to perennial ryegrass-white clover; to compare the effect of time of day and N fertiliser inputs on the chemical composition of alternative forages which may prove useful in reducing the supply if N to the grazing animal and; to investigate management strategies around time of day allocation which allow for at least 50% of the animals diet to be plantain.

Specific objectives were:

1. To determine milk production and composition, urine and faecal composition and urine output of dairy cows in early and late lactation grazing plantain and compare that with perennial ryegrass-white clover;

2. To determine the seasonal nutritive value of traditional pasture species (perennial ryegrass and white clover) compared with alternative forages (chicory, plantain, Italian ryegrass) and determine management strategies (diurnal allocation and reduced N fertiliser inputs) which would reduce the N concentration of the forage and/or improve the nutritive value;

3. To determine seasonal distribution of secondary plant metabolites in plantain herbage and identify potential management strategies to enhance their concentrations.

1.3 Hypotheses

Plantain will provide a suitable forage for milk production whilst reducing urinary N losses from dairy cows. Afternoon harvesting of forages will result in an increase in WSC at the expense of CP.

1.4 Thesis structure

This thesis is presented in eight chapters, including this introduction (Figure 1.1). In Chapter 2, literature concerning current N leaching issues from typical dairy systems, previous mitigation strategies and the potential of plantain to be used as a forage from milk production and reduced urinary N is discussed. Chapter 3 reports on a study conducted over two grazing experiments which compares milk production, urine and faecal composition and total urine output from cows grazing perennial ryegrass-white clover, plantain and a spatially separated 50-50 perennial ryegrass-white clover and plantain diet (Objective 1). Chapter 4 aims to characterise the diurnal change in chemical composition of alternative forages in a plots experiment (Objective 2). This is supported by an investigation of rumen degradation characteristic of one of the forages (plantain) harvested diurnally are compared to the rumen degradation characteristics of diurnally harvested perennial ryegrass-white clover in Chapter 5 (Objective 2). In Chapter 6 the results of Chapter 4 and Chapter 5 are used to design a diurnal management strategy which provides a 50% perennial ryegrass-white clover and 50% plantain diet. Chapter 7 investigates the secondary metabolite composition of plantain and the effects of time of day and N fertiliser on metabolites. Finally, in Chapter 8 the results are drawn together and further research areas are suggested.

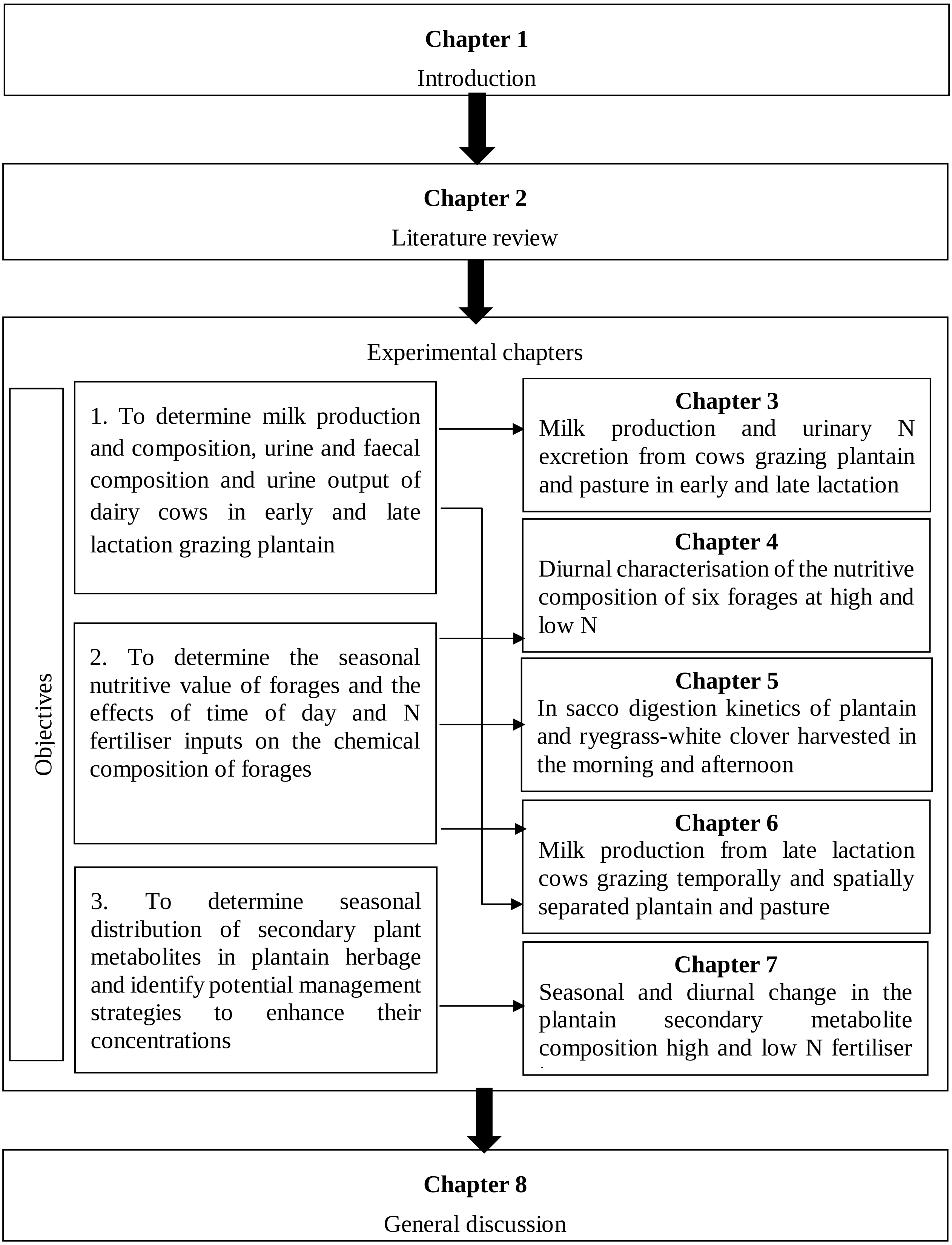


Figure 1.1 Diagrammatic representation of the structure of the thesis and the relationship of each chapter to the general aim and main objectives of the research presented in this thesis.

Chapter 2

Literature review

Over the past 20 years the New Zealand dairy industry has expanded dramatically. Dairy cattle in the national herd increased from 3.4 to 5.9 million from 1990 to 2010 with the bulk of expansion in the Canterbury region. Prior to this, mixed cropping rotations were the dominant agricultural systems in the area. The temperate maritime climate allows for grazing outdoors year round with only limited supplementary feeding necessary in the winter months. In the last decade famers have been aiming for increased production both in dairy and meat production (Edwards *et al.* 2007; Golding *et al.* 2008). This has led to a focus on increasing the productivity and quality of pastures used for grazing ruminants. Issues arise in nitrogen (N) loading from animals grazing pastures. Environmental impacts of the dairy industry in New Zealand have become a key focus with increasing interest surrounding N leaching issues (Bryant *et al.* 2007; Ellis *et al.* 2011). Regional Councils throughout New Zealand have been developing regulations that place a limit on the amount of nitrate-N leaching from agricultural land. These regulations may require substantial reductions in nitrate leaching from current dairy farm levels, and mitigation measures are sought. To maintain New Zealand's competitive advantage grazed forages will continue to be the primary source of feed for grazing dairy animals. Mitigation strategies that continue to use easily managed forage, as a primary feed source are required; these strategies should not be at the expense of production.

2.1 Perennial ryegrass-white clover pastures

Milk solids production and profitability of New Zealand dairy farms is tightly linked to the amount of herbage harvested by the grazing animal (Holmes *et al.* 2007). The supply of feed for dairy cows the single largest component of dairy farm operational costs. The primary source of home grown feed is grazed perennial pastures. The majority of dairy systems in New Zealand use perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) pastures which are normally grown in a simple binary mixture (Charlton *et al.* 1999).

The predominant use of perennial ryegrass-white clover pastures reflects desirable characteristics of these species. The pasture is easy to establish, contributes a high herbage dry matter (DM), has complementary growth patterns and is tolerant of a wide range of environments and grazing management (Kemp *et al.* 1999). A typical perennial ryegrass-white clover pasture under irrigation on a Canterbury dairy farm can produce 21 t DM/ha/yr (DairyNZ 2017). However there are some limitations to perennial ryegrass-white clover pastures.

Production of perennial ryegrass-white clover pastures is highly dependent on N fertiliser inputs and supplementary irrigation. Further, pasture quality can be low in perennial ryegrass-white clover in late spring and early summer (Burke *et al.* 2002). The pasture is also susceptible to the attack by a range of insect pests including grass grub (*Costelytra Zealandia*) and Argentine stem weevil (*Listronotus bonariensis syn Hyperodes bonariensis*) (McFarlane 1990). These limitations can result in reduced feed supply leading to sub-optimal dietary intake and reduced milk production, accelerating the rate of decline in post peak milk yield (Holmes *et al.* 2007). Of particular concern is the role perennial ryegrass-white clover in N losses from a system. Reliance on N fertiliser inputs contribute to a high protein content of perennial ryegrass-white clover pastures which are above the requirements of the grazing animal. Nitrogen in excess of an animals requirements is excreted predominately in the urine where it is easily leached through the soil profile and into waterways.

2.2 Nitrogen

Nitrogen in pasture-based livestock grazing systems is a challenging nutrient to manage. It transforms between chemical states dependent on environmental conditions (moisture, temperature, humidity) and soil composition. The N cycle (Figure 2.1) represents important processes in the soil, plant and atmosphere systems, whereby N is transferred from one form to another (McLaren *et al.* 1996). Under grazing N moves from pastures to fresh water in the form of nitrate (NO_3^-). As an anion, NO_3^- does not bind to soil particles. It remains in the soil solution and is subsequently leaches when drainage occurs. This is an environmental concern. Elevated NO_3^- concentrations in underground and surface water bodies can cause eutrophication which may impose harmful effects to aquatic ecosystems (Di *et al.* 2002a). A NO_3^- concentration in drinking water which exceeds 11.3 mg N L^{-1} is deemed unsafe for human consumption because it may lead to methaemoglobinaemia (blue baby syndrome) in infants.

FIGURE REMOVED FOR COPYRIGHT COMPLIANCE

Figure 2.1: The Nitrogen cycle: Inputs, transformations and outputs (McLaren *et al.* 1996)

Inputs of N to the dairy farm system (through N fertiliser, biological fixation and supplementary feeds) are greater than outputs (in milk, animal or feed sale) from the system. Only 25-30% of the N eaten by cows is retained for milk protein or other animal needs. The rest is excreted in dung (about 25% of total N eaten) or urine (about 50% of total N eaten) (Pacheco *et al.* 2008). It is the N in urine that is the main problem for freshwater quality in agricultural catchments (Ledgard *et al.* 2007). Urinary N deposition (predominately in the form of urea) occurs at rates which usually far exceed the capacity of plants to assimilate that N for their own use. On contact with the soil urinary urea is hydrolysed to ammonia (NH_3) which is nitrified by soil bacteria to produce NO_3^-. Nitrate, as an anion, is easily leached through the soil profile when drainage occurs. The small scale of urine patches is a major issue, resulting in N loading at levels up to 800 kg N/ha (Di *et al.* 2001; Di *et al.* 2002a). The higher the concentration of N in the diet of the animal, the higher the concentration in the urine and the greater the risk of nitrate leaching (Castillo *et al.* 2000).

Regional Councils across New Zealand are currently setting nutrient limits for groundwater, lakes and rivers in their catchments, as required by the NZ Government under the National Policy Statement on Freshwater Management. These nutrient plans will likely require dairy farmers to reduce N losses in several catchments. In these situations, farm systems and/or management will have to change. It is important that any mitigations strategies minimise any negative changes on milk production and farm profitability.

2.3 The urine patch

2.3.1 Urine composition

The concentration of N in the urine of ruminants varies from less than 1 to over 20 g N/L (Whitehead 1995; Dijkstra *et al.* 2013). A *meta*-analysis by Selbie *et al.* (2015) of published data found the average urinary N concentration of dairy cattle grazing grass pastures to be 6.9 g N/L. However there can be a large variation between animals, even when grazing the same pasture type.

Nitrogen in urine is contained in a number of N compounds. Urea is the largest proportion of urinary N. Nitrogen also exists in the urine in allantoin, hippuric acid, creatine, creatinine and ammonia. The proportion of each compound can change depending on the diet of the animal. Typically, as N intake increases, the proportion of urinary N present as urea increases (Petersen *et al.* 1998). According to Jarvis *et al.* (1995) urea contributes 90% of urinary N for cows grazing heavily fertilised grass based pastures. The proportion of total N as urea can show diurnal variation, with greater proportions in the morning than evening (Petersen *et al.* 1998; Bryant *et al.* 2013).

There are two main factors which influence urinary N. The first is N intake which drives the amount of surplus N relative to an animal's requirement. Secondly, water intake effects urinary N concentration due to its influence on both volume and frequency of urination. There is a linear relationship between N intake and urinary N output with increased N intake resulting in greater N losses via urine. However, increased N intake does not necessarily relate to increased urinary N concentration. This is because there tends to be an increase in water intake for animals grazing higher N diets, which in turn results in greater urine volumes thereby diluting urinary N concentration but increasing overall urinary N output. This has been seen by Van Vuuren *et al.* (1997) who reported a 74% increase in urination volume following a change from low to high N diets with only a 0.2 g N/l increase in urinary N concentration. This highlights the importance of understanding not only the urinary N concentration, but also the amount of urine produced.

2.3.2 Urine volume and frequency

Urination output in terms of volume is influenced predominately by water intake. Water intake is related to the mineral load ingested and excreted by the animal. This means that pasture species which have differing mineral content will likely alter water intake of an animal and thereby alter urine volumes. Urination volume and frequency is known to be highly variable between individual animals and with time of day (Betteridge *et al.* 1986). A review by Selbie *et al.* (2015) showed that on average dairy cows excreted 2.1 L per urination. However, this can be highly variable ranging from >0.5 L to >8 L per urination (Betteridge *et al.* 2013). Further, urine output in grazing dairy cows is rarely measured, possibly due to the labour and cost requirement for urine capture in the paddock. There is less variation in the frequency of urinations across a day. Typically cattle have 10-12 urination events per day (Selbie *et al.* 2015).

The volume and frequency of urinations have implications on the loading and spread of N to the paddock. The amount of N deposited on the soil (N loading rate) at a urine patch is a function of the N concentration of the urine, the urine volume excreted during a urination event and the size of the urine patch:

$$N\ loading\ (kg\ N/ha) = urine\ N\ conc.\ (g\ N/L) \times \frac{Volume(L)}{Surface\ area\ (m2)} \times 10$$

Selbie *et al.* (2015) calculated urine N loading from dairy cows to averages 613 kg N/ha. This is much lower than the common cited reference of N loading from Haynes *et al.* (1993) who assumed an average N loading of 1000 kg N/ha. Their value consisted of a higher than average urine N concentration of 10 g N/L, urination volume of 2 L and urine patch surface area of 0.2 m^2.

Reductions in urinary N concentration would be beneficial to reduce N loading provided increases in urine volume without increasing urine patch size do not occur. With larger urination volumes it is likely an increase in patch surface area would increase thereby reducing N loading. This outlines the importance of experimental work measuring not only urinary N concentration but also urine output in terms of total litres produced, litres per urination and urination frequency across a day. It could be favourable if there were an increase in urination frequency with reduced urine N concentration to provide a better spread of urine across a paddock with overall reduce N loading.

2.4 Approaches to reduce nitrate leaching

Nitrogen leaching losses can be reduced in several ways including: (1) increasing the utilisation of N in the cow, (2) reducing the use of N inputs (fertiliser), (3) changing the diet of the animals to reduce the N intake of animals or (4) inhibiting the change in N form in the soil. Management practises such as reducing animal numbers or time on pasture can also be utilised but are not always an efficient way of reducing nitrate leaching. Alternative practices need to consider how effective the strategy is for reducing N losses, how much production risk is involved and any possible consequences for profitability.

There have been developments such as the use of dicyanamide (DCD), a nitrification inhibitor that reduces the rate of transformation of ammonia to nitrate and therefore keeps urinary N in a less leachable form. This proved to reduce nitrate leaching under urine patches by 20-60% depending on soil type (Di *et al.* 2002b). DCD could have been an efficient easy way to reduce the risk of N leaching to the dairy system. In 2013 the product was suspended from sale in NZ due to detection of DCD in NZ milk products. The future commercial availability of DCD is uncertain. Alternative solutions are sought.

The largest contributor to nitrate leaching in a dairy grazing system is the urine patch. A reduction in urinary N output can result in a reduction in N leaching. A positive relationship between N intake and urinary N losses has been well established (Tas *et al.* 2006b). Predictions of N losses are primarily based on N intake. The inefficient use of N in animals grazing perennial ryegrass-white clover pastures is due to an over-supply of protein in the pasture (Pacheco *et al.* 2008). Lactating dairy cows have a crude protein requirement in their feed in the order of 15 - 18% in early lactation and 12 - 15% later in the season (CSIRO 2007). Total dietary CP concentrations of greater than 20% are surplus to requirements. Excess protein is converted to urea and excreted contributing to nitrate leaching and nitrous oxide emissions. A reduction in N intake has reduced N output. Restricting fertiliser N inputs to achieve this can result in losses in pasture production and subsequent milk production so this is not an efficient method to reduce leaching losses.

2.4.1 Diurnal reduction in CP

An increase in WSC composition in perennial ryegrass may be beneficial to livestock systems. Grasses which have been bred for increased WSC concentration in the U.K. have been extensively tested and shown inconsistent reductions in urinary N concentration (Miller *et al.* 2001; Moorby *et al.* 2006; Tas *et al.* 2006b). While Miller *et al.* (2001) showed a higher WSC concentration in high sugar ryegrass reduced N output by 28.7 g/d compared with perennial ryegrass, Tas *et al.* (2006b) found no difference in urinary N concentration from the two ryegrass cultivars. Further, the expression of the high WSC trait is inconsistent in New Zealand as the climatic conditions do not meet the temperature requirements for expression of the high WSC trait (Smith *et al.* 1998; Parsons *et al.* 2004; Cosgrove *et al.* 2009). However, the use of a diurnal change in plant composition has shown has shown a consistent increases in WSC concentration from morning to afternoon (Miller *et al.* 2001). Miller *et al.* (2001) showed that perennial ryegrass harvested in the afternoon had a 30% greater WSC concentration than that harvested in the morning (Figure 2.2). Therefore in a New Zealand situation, utilising diurnal management strategies to increase WSC of forages may be beneficial to grazing systems.

FIGURE REMOVED FOR COPYRIGHT COMPLIANCE

Figure 2.2: Mean WSC concentrations of snip samples of high sugar (-◊-) and control (▪) grasses over 3 consecutive days in August with a min temp. of 13^{o}C and a max temp. of 19^{o}C (Miller *et al.* 2001).

Edwards *et al.* (2007) produced a comprehensive review of high sugar ryegrass in dairy systems compiling results from various experiments to show that the proportion of WSC to CP was more important than the concentration of WSC alone. A WSC:CP ratio of above 0.7 led to a decrease in the proportion of N intake excreted from the dairy animal. A reduction in urinary N concentration could be achieved by either increasing the WSC concentration or by decreasing the CP content in the pasture, however the two scenarios have different effects on digestibility and subsequently milk yield (Ellis *et al.* 2011). The increase in WSC has been achieved by harvesting pasture in the afternoon to exploit the diurnal changes in plant composition (Moorby *et al.* 2006). Considering this, characterising pastures that are high in WSC at the expense of CP, would be favourable to identify forages that could reduce N losses in a New Zealand farm system. Whilst the WSC:CP has been well studied for perennial ryegrass, less information is available on alternative forages such as herbs. Characterising the diurnal influence on nutritive value of alternative forages will be explored in Chapter 4 of this thesis.

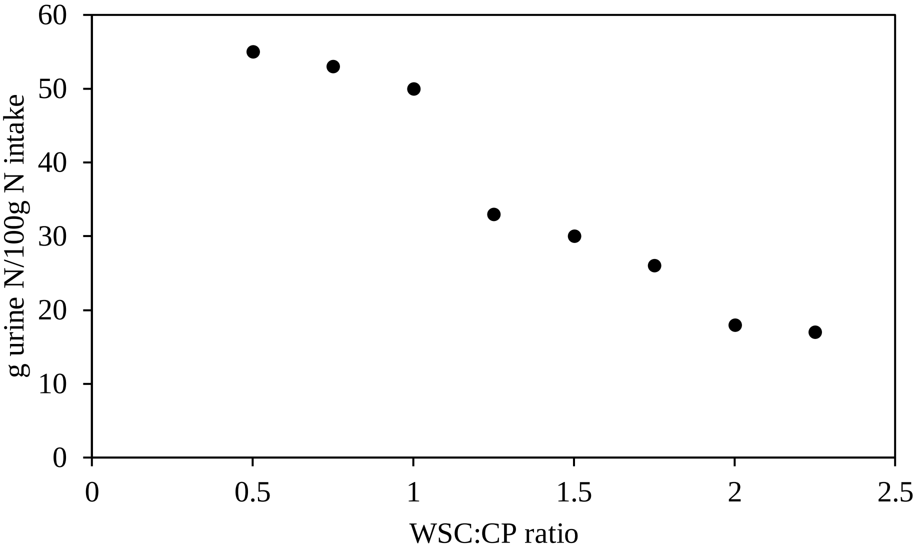


Figure 2.3 Combined data for a range of UK and Dutch studies showing a continuum between N utilisation efficiency for urine in dairy cows, in relation to WSC:CP ratio of the forage component of the diet offered (Edwards *et al.* 2007)

2.4.2 Alternative pastures for reduced urinary N

Due to the inefficient use of N in systems which use perennial ryegrass-white clover pastures, alternative forages have grown in popularity to combat environmental issues whilst improving or maintaining production. Diverse pastures where additional legumes and herbs are added to standard ryegrass-white clover swards have shown an advantage in not only yield over the summer period but also in the mitigation of nitrogen leaching issues due to lower N concentrations compared with perennial ryegrass-white clover (Nobilly *et al.* 2012; Woodward *et al.* 2012; Totty *et al.* 2013; Woodward *et al.* 2013).

DM production

The bimodal distribution of forage DM production from perennial ryegrass-white clover pastures creates challenges in maintaining high levels of milk production from a predominantly pasture based diet (Rawnsley *et al.* 2007). Periods of over- and under-supply of forage are addressed through forage conservation and the use of supplements. However, inefficiencies in forage conservation and feeding of conserved forage along with substitution of pasture with concentrates (Wales *et al.* 2006) means that these practices are less efficient than direct grazing.

This can lead to a reduction in the total amount of pasture that can be consumed thereby limiting milk production. Therefore the increased summer production from alternative forage species may be beneficial to New Zealand systems by providing a better supply of pasture throughout lactation.

Table 2.1 Experiments which compared annual DM production of perennial ryegrass-white clover and diverse pastures where at least 3 alternative forage species were added in a mix with perennial ryegrass and white clover

Study	Location	Additional species	Difference in DM production
(Woodward *et al.* 2013)	Waikato, New Zealand	Prairie grass, lucerne, chicory and plantain	None
(Nobilly *et al.* 2012)	Canterbury, New Zealand	Prairie grass, red clover, chicory and plantain	+10%
(Tharmaraj *et al.* 2008)	South West Victoria, Australia	Red clover, chicory	+15%
(Tharmaraj *et al.* 2014)	South West Victoria, Australia	Cocksfoot, red clover, chicory	+9%

Diverse pastures have shown the ability to increase dry matter production over standard ryegrass-white clover pastures particularly during dry season. Under irrigation the inclusion of herbs and additional legumes to perennial ryegrass-white clover based pastures has improved annual DM and ME production of dairy pastures. When simple two-species grass (perennial ryegrass or tall fescue-white clover pastures were compared with diverse pastures where herbs (chicory and plantain), legumes (red clover and lucerne) and prairie grass were added to the simple mixtures no difference in crude protein content was found (Nobilly *et al.* 2012). The average annual herbage DM production over two years was 1.62 t DM/ha greater in diverse (16.77 t DM/ha) than simple (15.15 t DM/ha) pastures. The authors concluded that diverse pastures containing additional herbs and legumes may play an important role maintaining or increasing milksolids production per cow and per ha.

A similar experiment in the Waikato compared DM production of perennial ryegrass-white clover with and without additions of prairie grass, lucerne, chicory and plantain over 3 years (Woodward *et al.* 2013). While there was no difference in annual DM production between the simple and diverse pastures (averaging 15 t DM/ha/y), the diverse pasture offered the benefit of a greater supply of herbage in the summer, particularly in drought years. This summer or drought advantage in DM production has been seen consistently in experiments where additional legumes and herbs are included in a mix with perennial ryegrass-white clover (Table 2.1) (Daly *et al.* 1996; Tharmaraj *et al.* 2008; Nobilly *et al.* 2012; Tharmaraj *et al.* 2014). In the experiment by Nobilly *et al.* (2012), the greater annual DM production from diverse pastures was due to a 1 t DM/ha advantage in summer.

Urinary N effects

Diverse pastures potentially offer a strategy to reduce the environmental footprint of dairy farming. Diverse pastures may reduce nitrate leaching through affecting the amount and concentration of N excreted in urine. Initially experiments which used alternative plant species to reduce urinary N excretion focussed on the role of secondary plant compounds, particularly tannins, in altering partitioning of N within the cow between urine and faeces (Woodward *et al.* 2000). The role of tannins as a potential to alter the partitioning of N away from urine and towards milk has been discussed and defined as a feasible option (Mueller-Harvey 2006). However, tannins are primarily found in plant species (e.g. birdsfoot trefoil and *Lotus penduculatus* L.) that are poor competitors in mixtures and difficult to manage under grazing (Jones *et al.* 1988). More recently there has been a move to examine the effects of including herbs; chicory and plantain, on urinary N.

In a metabolism stall study, Woodward *et al.* (2012) measured N partitioning in cows fed either perennial ryegrass–white clover pasture or a pasture that contained chicory, plantain and lucerne in addition to perennial ryegrass-white clover. Both urinary N concentration and urinary N output were lower from cows fed the diverse forage than from those fed the perennial ryegrass–white clover sward (2.6 g versus 6.2 g N/L and 100 versus 200 g N/cow/day, respectively). This may have been due the total N intake of the cows (350 versus 466 g N/cow/day), reflecting the well-defined relationship between N intake and urinary N output (Kebreab *et al.* 2001).

Table 2.2 Urinary N concentration (%) and milk production (kg/cow/day) from cows grazing perennial ryegrass-white clover (simple) pastures or those with additions alternative pasture species (always including chicory and plantain) from a range of experiments carried out in New Zealand

	Urinary N concentration (%)			Milk production (kg/cow/day)		
Study	Simple	Diverse	Significant	Simple	Diverse	Significant
(Totty *et al.* 2013)	0.57	0.34	Y	1.55	1.47	N
(Woodward *et al.* 2012)	0.62	0.26	Y	1.03	1.16	Y
(Bryant *et al.* 2017)	0.42	0.29	Y	1.70	1.79	N
(Edwards *et al.* 2015)	0.61	0.49	Y	2.09	1.94	N

The effects of diverse pastures on urinary N concentration has also been examined in grazing experiments in New Zealand. Urinary N concentration was reduced by at least 20% in all these experiments by offering cows a diverse pasture which included perennial ryegrass, white clover with additional of chicory and plantain (Totty *et al.* 2013; Edwards *et al.* 2015; Bryant *et al.* 2017). Unlike the confinement feeding study by Woodward *et al.* (2012), a grazing experiment by Totty *et al.* (2013) found that pasture N intakes were similar from diverse (550 g N/cow/day) and simple pastures (589 g N/cow/day). The mechanisms behind the reduced concentration of urinary N excreted in the grazing experiments are unclear as they did not reflect just reduced N intake by cows. It is possible the added species may improve the nutrient synchrony of the pasture (with particular reference to metabolisable protein), act as a diuretic, and with the lower DM% in these forages, increase water intake, and, hence, urination volume may be higher. This was shown by Beukes *et al.* (2014) who collated data from experiments which measured urine volumes, DMI and feed DM% and derived a linear relationship between water intake in the feed and urine volume (Figure 2.4).

FIGURE REMOVED FOR COPYRIGHT COMPLIANCE

Figure 2.4 Predictive equation for urine volume per cow per day using amount of ingested water, derived from dry matter (DM) intake and feed DM% (Beukes *et al.* 2014).

Regardless of the mechanism behind increased urine volumes, the results compiled by Beukes *et al.* (2014) represent an opportunity to increase the spread of urine patches within a pasture and, in addition, lower the N concentration in each patch. The lower N concentration observed under diverse pastures should lower N loading at the urine patch and increase the fraction of urinary N that is captured by the plants before it is leached or lost to the atmosphere (Di *et al.* 2007). When Beukes *et al.* (2014) modelled the potential of diverse pastures to reduce leaching at the whole of farm scale, they found a reduction of 11% and 19%, where 20% and 50%, respectively, of the farm area was sown to diverse pastures. However it is important these reductions do not impede production and profitability of the farm.

Milk production

Maximising milk production whilst maintaining adequate body condition of dairy animals is key goal of dairy production systems. Pastures which help to achieve this goal should be utilised. The effects of diverse pastures on milk production in dairy grazing systems have given mixed results (Table 2.2). Woodward *et al.* (2012) compared milk production of 16 cows fed either a perennial ryegrass pasture or diverse pasture mix which also contained chicory, plantain and lucerne in an indoor environment. Despite cows on the diverse pasture having a reduced DM intake (14.6 kg DM/cow/day) compared with the perennial ryegrass-white clover cows (15.8 kg DM/cow/day) milk solid production was increased (1.16 and 1.03 kg/cow/day respectively). In contrast, Totty *et al.* (2013) found no difference in milk solid production in autumn for late lactation cows grazing perennial ryegrass-white clover, high sugar ryegrass-white clover and diverse pastures which contained chicory and plantain. Similar results have been found by Edwards *et al.* (2015) and Bryant *et al.* (2017). Conclusions from these studies were that the diverse pastures maintained milk production at the same levels with cows that grazed perennial ryegrass- white clover with the greatest advantage being the reduction of urinary N to the environment.

Limitations of diverse pastures

Whilst forage diversity in a pasture has shown benefits to grazing dairy systems through increased pasture production in dry seasons (Daly *et al.* 1996; Nobilly *et al.* 2012; Woodward *et al.* 2013) and reduced urinary N concentrations (Totty *et al.* 2013; Edwards *et al.* 2015; Bryant *et al.* 2017), several species sown together in a mixed sward may be difficult to manage. Pasture persistence has been identified as an important factor to farmers when considering which forages to sow (Tozer *et al.* 2011). Several experiments have identified chicory as a poor performer in long term pasture swards (Neal *et al.* 2009; Tozer *et al.* 2011; Tozer *et al.* 2017). For example Tozer *et al.* (2017) found that a diverse pasture sown with perennial ryegrass, white clover, chicory and plantain maintained just 1% basal cover of herbs four years after sowing, compared with 11% in the first year of growth. Further the proportion of weeds increased from 5% to 24% from year 1 to year 4. Sanderson *et al.* (2005) suggested that farmers would be required to re-establish chicory frequently in mixed pasture swards to maintain presence of the species. The authors also stated that it may be of greater benefit to identity key species present in the mixture that are a driver of herbage yield advantages rather than maintain a highly diverse mix. Of the species usually present in diverse pasture species plantain has been shown to be highly competitive and persist longer than chicory (Tozer *et al.* 2011). For example, in the field measurements Tozer *et al.* (2011) found plantain, when sown in a mix, was observed in 100% of 1 to 15 years old paddocks in Waikato and in 11 of 12 of 1 to 10 year old paddocks in Canterbury. Plantain may, therefore, provide high quality forage for many years when sown as part of a sown pasture mix. In contrast, the authors reported that most farmers who had grown chicory, had only a small proportion of the forage remaining in rotationally grazed dairy pastures 3-4 years after sowing. Further, some of the dairy farmers interviewed commented that commercial plantain cultivars appeared to be spreading around the farm via dung. This is likely, as 58% of plantain seeds germinate after being ingested by and passing through cattle (Hammond 1982).

Weed control can be difficult in diverse pastures containing broad leaf species. Few herbicide manufacturers have information for safe herbicides when plantain, chicory and clovers are grown together as a mix. Gawn *et al.* (2012) investigated the effects of herbicides on pasture containing a mix of plantain, chicory and clovers. None of the herbicides with activity against broad-leaved weeds were tolerated well by all four pasture species (Table 2.3). Paraquat/diquat showed good control of weeds with no hemlock or nightshade present after 7 weeks and only 2.8 kg DM/ha of dock, however chicory also was not present in the pasture after the application of paraquat/diquat (Table 2.3). The most promising was flumetsulam, which was tolerated well by the chicory and clovers (1200 and 57 kg DM/ha respectively 7 weeks after application), but plantain suffered a severe initial check in growth. Of the other herbicide treatments, 2,4-DB and diflufenican were too damaging to both the plantain and chicory, while diuron and bentazone were too damaging to chicory. This experiment shows the complication in finding a suitable herbicide for diverse pastures. However, if these species were grown as a monoculture management of weed species could be much less challenging.

Table 2.3 Dry matter (kg DM/ha) of sown species (plantain, chicory and clovers) and weeds (hemlock, dock and nightshade) 7 weeks after herbicide application (Gawn *et al.* 2012)

	Sown Species			Weeds		
	Plantain	Chicory	Clovers	Hemlock	Dock	Nightshade
Control	88	82	3	351	773	247
Haloxyfop	86	147	3	620	358	620
Flumetsulam	42	1200	57	40	46	244
2,4-DB	46	178	28	1006	22	0
Diuron	1445	21	25	210	151	0.1
Bentazone	1008	10	52	0.2	53	94
Paraquat/diquat	1108	0	13	0	2.8	0
LSD (P=0.05)	360	446	42	329	303	261

2.4.3 Next steps

The use of diverse pastures or diurnal management strategies in the literature recognises the use of pastoral systems is unlikely to change in the New Zealand dairy industry. A gap in information exists in the characterisation of individual plant species for reducing the N concentration consumed by grazing dairy animals. Combining any beneficial effects of individual plant species with management that optimises any beneficial diurnal changes in plant composition could help to reduce nitrogen output and subsequent leaching on dairy farms. This approach recognizes that the main source of nitrate leaching is the urine patch. Utilising plants with lower N concentration could reduce N excretion in urine by reducing the N content of the feeds they are eating and in pastoral systems. Recognising a single species capable of achieving a reduction in N in the urine would be most beneficial. Of the species present in diverse pasture, plantain appears to be the most likely candidate to cause a reduction in urinary N. Plantain is known to contain secondary plant metabolites (Stewart 1996; O'Connell *et al.* 2016). These metabolites may be influencing N partitioning through the animal.

2.5 Plantain

Plantain, also known as ribwort plantain, belongs to the Plantaginaceae family (Stewart *et al.* 2014). Plantain is an upright perennial herb, which tolerates many pests and diseases. Plantains have white, many flowered seed heads which are compact and cylindrical on the end of the stem. Until recently plantain was regarded as a weed and thought to be of little use to agricultural systems (Levy 1970). Common weed species of plantain grow close and flat to the ground and are characterised small rounded leaves with very low winter growth and low summer growth (Stewart 1996). Plantain is native to Eurasia (Webb *et al.* 1988). In New Zealand there are two main cultivars of plantain used in pastures, Ceres Tonic and Grasslands Lancelot. Grasslands Lancelot is the result of four generations of selective breeding where the best plants were taken from 109 seed populations based in the North Island (Stewart 1996; Rumball *et al.* 1997). Grasslands Lancelot has semi-erect leaves but requires a lax grazing management to maintain a relatively high residual (>1600 kg DM/ha) (Charlton *et al.* 1999). The cultivar has a high tiller count, with medium to large leaves. Grasslands Lancelot is winter dormant but has high growth in summer (Stewart 1996). Ceres Tonic plantain was selected from seeds originating in Portugal. The cultivar was chosen for its characteristic of maintaining upright leaves even under hard grazing (Stewart 1996). Ceres Tonic also has greater winter growth compared with Grasslands Lancelot, though has a lower number of tillers. Ceres Tonic, when sown with perennial ryegrass, increases dry matter which means it has the potential to maintain and improve production (Moorhead *et al.* 2009). This thesis will focus on the properties of Ceres Tonic.

Ceres tonic plantain has become increasingly popular as a specialist crop or sown in a pasture mix (typically at ~2 kg/ha). Plantain is regarded as a ‘low fertility’ plant and soil fertility determines the competitive ability of plantain when sown with other grasses. It establishes rapidly and grows on a wide range of soil types with varying soil acidity (pH 4.2-7.8) and fertility (Charlton *et al.* 1999). Plantains coarse root system consists of both a tap root and fibrous roots. The taproot grows to a shallower depth than other tap rooted plants such as chicory and lucerne.

2.5.1 DM production

Plantain has been shown to produce greater DM in the summer and autumn compared with grass based pastures, which allows a greater distribution of DM yield throughout the year (Moorhead *et al.* 2009). This is due to plantains drought tolerance and considerable summer heat tolerance (Stewart 1996; Rumball *et al.* 1997; Cranston *et al.* 2016).

In a mix with perennial ryegrass and white and red clover, plantain, provided significant dry matter production advantages which ranged from 6 t DM/ha in the first year of growth to 1.2 t DM/ha by Year 3 (Moorhead *et al.* 2009). Plantain contributed between 32-90% of the yield. The yield advantage of plantain-based pastures over perennial ryegrass-based pastures were due to greater yields in summer (1.8 t DM/ha) and autumn (0.9 t DM/ha) Figure 2.5. The authors concluded that adding ‘Tonic’ plantain to perennial ryegrass-white clover pastures, even at low seeding rates (2 kg/ha), has the potential to increase production levels and to improve dry matter distribution over time. Although the difference declined over time there was still a real advantage to plantain-based pastures by Year 3.

FIGURE REMOVED FOR COPYRIGHT COMPLIANCE

Figure 2.5 Mean seasonal distribution of dry matter production on four farms over 3 years from perennial ryegrass-based pastures and ‘Tonic’ plantain-based pastures in Northland. Bars represent least significant difference (P<0.05). From (Moorhead *et al.* 2009).

Similar results have been observed in other studies with pastures containing plantain consistently showing increased summer growth when productivity of perennial ryegrass-white clover can restrict livestock production (Stewart 1996; Powell *et al.* 2007; Nobilly *et al.* 2012). Powell *et al.* (2007) compared DM production of plantain, chicory and red clover at 8, 12 and 19 weeks after sowing. Plantain produced more DM than chicory in the first year (17 vs. 14 t DM respectively). Herbage accumulation (kg DM/ha) at 8, 12 and 19 weeks was higher for plantain than chicory and red clover (Table 2.4). Grazing 8 weeks after sowing resulted in >30% plant losses for all the 3 species. However, fewer losses (3%) for plantain were recorded when compared with chicory (13%) at 19 weeks grazing after sowing.

Table 2.4 Dry matter production (kg/ha) of plantain, chicory and red clover just before first grazing times at 8, 12 and 19 weeks after sowing (Powell *et al.* 2007)

	DM production (kg DM/ha)			SEM
Species	8 weeks	12 weeks	19 weeks	Grazing time
Plantain	849	2606	5214	158.5
Chicory	280	1465	3026	
Red clover	436	2049	2936	
SEM species	152.0			

Results from these experiments (Powell *et al.* 2007; Moorhead *et al.* 2009) suggest plantain has a potential to produce at least as much DM as perennial ryegrass-white clover pastures with the potential of a more even seasonal distribution of DM supply. The bimodal distribution of forage DM production from perennial ryegrass-white clover pastures can challenging challenges in maintaining high levels of milk production from a predominantly pasture based diet (Rawnsley *et al.* 2007). There is considerable potential for plantain to supply high quality forage throughout the year which would support milk production of dairy cows.

2.5.2 Animal performance

Dry matter production is not the only consideration when determining if an alternative forage may be suitable in a grazing situation. The forage also needs to support livestock production. Plantain may be suitable to support milk production in grazed dairy system, however, there is currently little research to support this. Plantain has been tested in sheep production systems in New Zealand. Several experiment have confirmed plantain combined with red and white clovers, with or without chicory will support lamb live weight gains of 250-350 g/day at high stocking rates (Golding *et al.* 2008; Kemp *et al.* 2010). In the experiment by Kemp *et al.* (2010), animals on plantain and clover mix produced 720 kg/ha of net carcass weight in a year compared with 400 kg/ha on perennial ryegrass and clover pastures. Further, plantain in a mix with red and white clover and chicory has increase ewe milk production and ewe and lamb live weight gain in spring (Hutton *et al.* 2011). At 66 days after lambing Hutton *et al.* (2011) found lambs on the herb mix weighed on average 20.67 kg compared to 17.55 kg for lambs on perennial ryegrass-white clover. The attributed the increased liveweight to greater ewe milk production, with ewes consistently producing around 30% more milk.

Ease of prehension has allowed increased DM intakes of weaned lambs offered herbs or legumes (Fraser *et al.* 1996a). Kenyon *et al.* (2010) also found faster lamb liveweight gains in early lactation, and increased survival of Romney lambs on an herb-clover mix. These results could be due to either, increased ewe milk production, altered milk composition, increased lamb herbage intake, or a combination of these factors. Regardless of the mechanisms involved, the literature reviewed here shows a consistent advantage of including plantain in an animal's diet on livestock production. However there is little information on the effects of plantain on milk production from dairy cows. Those experiments which examined the effects of plantain on milk production from cows only used the forage in a mix (Woodward *et al.* 2012; Totty *et al.* 2013; Edwards *et al.* 2015; Bryant *et al.* 2017). A promising result from the experiments is that where plantain has been included in a mix, milk production was never negatively impacted. Therefore it can be hypothesised that feeding dairy cows a plantain based diet should at least maintain milk production which would be expected from a perennial ryegrass-white clover pasture.

2.5.3 Mineral compostion

According to Sanderson *et al.* (2003) plantain contains higher concentration of macro (N, P, K, Ca, Mg, Na) and micro (Co, Cu, Fe Mo, Se, Bo Mn, Zn, Cr, B) mineral than perennial ryegrass. However, estimates of the mineral content of plantain differs between conditions and experiments (Table 2.5; Table 2.6). Of the macrominerals, Ca and Mg appear to be consistently higher in concentration for plantain compared to perennial ryegrass (Table 2.5) (Wilman *et al.* 1993; Wilman *et al.* 1994; Pirhofer-Walzl *et al.* 2011). Microminerals Co, Cu, Se, Zn, and Bo are also higher in plantain than in perennial ryegrass (Table 2.6) (Hoskin *et al.* 2006; Pirhofer-Walzl *et al.* 2011).

Table 2.5 Macronutrient content (g/kg DM) of plant material from ryegrass and plantain

		Macronutrients (g/kg DM)					
Experiment	Forage	N	P	K	Ca	Mg	Na
(Wilman *et al.* 1994)	Ryegrass	25.1	3.80	29.9	5.80	2.10	2.20
	Plantain	25.0	3.30	29.1	14.0	2.40	1.70
(Wilman *et al.* 1993)	Ryegrass	39.1	5.42	42.4	6.40	2.56	3.30
	Plantain	36.5	4.54	43.8	20.1	2.39	7.20
(Pirhofer-Walzl *et al.* 2011)	Ryegrass	20.0	3.95	33.95	4.65	1.75	1.20
	Plantain	15.95	3.8	30.0	15.7	2.75	0.55

Minerals play an important role in metabolic disorders like milk fever. In an experiment by Judson (2008) lambs grazing Tonic plantain appeared to have elevated liver concentrations of Cu and Se at slaughter relative to ryegrass fed lambs. Similar effects have been reported previously (Moorhead *et al.* 2002).The ability of cows to mobilise Ca from the bone is affected by acid-base balance that involves the dietary cation-anion difference (DCAD) estimated as (Na + K)-(Cl + S) in the diet (Judson *et al.* 1998). Perennial ryegrass-white clover pasture contains high levels of K (>5mg/kg DM) which increases DCAD in lactating cows and can lead to hypocalcaemia (Sanderson *et al.* 2003). Given the linear relationship between the risk of milk fever and DCAD (DeGaris *et al.* 2008), the mineral composition of plantain may be beneficial to reduce DCAD, thereby reducing milk fever incidences. Rugoho *et al.* (2017) reported reduced DCAD for plantain and rape mix compared with ryegrass pasture (171 vs. 258 mEq/kg DM respectively).

Table 2.6 Micronutrient content (g/kg DM) of plant material from ryegrass and plantain

		Micronutrients (mg/kg DM)								
Experiment	Forage	Co	Cu	Fe	Mo	Se	Mn	Zn	Cr	B
(Pirhofer-Walzl *et al.* 2011)	Ryegrass	-	6.1	66.6	1.25	-	73.2	23.2	0.3	3.9
	Plantain	-	8.2	63.5	0.40	-	33.3	30.8	0.2	20.3
(Hoskin *et al.* 2006)	Ryegrass	0.35	9.3	1271	0.31	0.04	-	-	-	-
	Plantain	0.36	14.0	795	0.34	0.06	-	-	-	-

2.5.4 Secondary compounds

Plantain is known to contain secondary plant metabolites which may act as diuretics (Rumball *et al.* 1997). Plantain contains the iridoid glycosides aucubin and catalpol and phenylpropanoid glycoside acteoside (verbascoside). The herbage contains approximately 4.1 % aucubin in leaf DM (Stewart 1996). Aucubin stimulates both the removal of uric acid from tissues to blood and the excretion of uric acid from the kidneys. Catalpol is present in concentrations of 0.7-5.0% of the DM. Catalpol is the active diuretic principle of the fruit of *Catalpa ovata*. Acteoside can be present in high levels (>5% DM) within the leaves of plantain (Fajer *et al.* 1992). Similar to aucubin, acteoside is thought to have developed in plants as a defence mechanism against predation from insects and is also known for its antimicrobial (Andary *et al.* 1982; Chen *et al.* 2012), and antioxidant effects (Zhou *et al.* 1991).

The concentration of secondary plant metabolites in plantain can vary. Aucubin concentrations in plantain cultivars Grasslands Lancelot and Ceres Tonic increased in late spring to mid-autumn in an experiment in Japan (Tamura *et al.* 2002). On the other hand, catalpol appeared to be relatively un-responsive to environmental variations. From late autumn onwards the levels of aucubin steadily declined at a rate of 0.13 %/^{o}C in Grasslands Lancelot and 0.20 %/^{o}C in Ceres Tonic relative to a decline in air temperature from 14.7 ^{o}C to 10.7 ^{o}C. The authors suggested that aucubin accumulates in great concentrations when the air temperate is optimal for growth. Similar results were found by Bowers *et al.* (1992) who measured the concentration of aucubin and catalpol every fortnight from late spring to early autumn. They found a significant increase in the concentration of both aucubin and catalpol over the growing season with the exception of summer. In midsummer a reduction in concentrations was seen. This occurred when the air temperature was high (~25-30^{o}C) which again suggests that concentrations are highest when temperatures are optimal for growth. For acteoside, concentrations increased in spring harvested plantain from 1.5% DM to 4.1% in autumn (Tamura *et al.* 2002). There is currently limited data on the concentration of secondary plant metabolites for New Zealand grown plantain.

2.5.5 Potential diuretic effects of plantain

The secondary compounds, lower DM% and mineral load of plantain may cause diuresis. In an experiment by Deaker *et al.* (1994), lambs grazing plantain had significantly greater adjusted kidney and liver weights compared with those grazing chicory, ryegrass or white clover. Similar results were also found by Fraser *et al.* (1996b). The authors suggested this was due to the diuretic properties of plantain (Deaker *et al.* 1994; Fraser *et al.* 1996b). This was supported by the lower serum creatinine and urea of plantain relative to ryegrass lambs. Plantain lambs also appeared to drink more water than their counterparts grazing other pasture species. Therefore using plantain may increase the spread of urine, thereby diluting the rate of urinary-N and increasing plant uptake of N. Recently an experiment using sheep in metabolism crates produced evidence that plantain has a diuretic effect (O'Connell *et al.* 2016). Sheep fed freshly cut plantain produced 1.7 L more urine on the first day of the experiment compared with those on ryegrass- white clover diets and 0.5 L more on 5 subsequent collection days. There are no known experiments which measure urine output of animals grazing plantain in situ.

Other diuretic feed sources have been successfully used to reduce the N concentration of urine from cows. Using salt as a diuretic an experiment in the Waikato, urinary N concentration of stall fed dairy cows offered pasture silage with 400 g salt/day was less than half the concentration (3.0 g N/L) of a control group given no salt (9.6 g N/L) (Ledgard *et al.* 2007). There were no adverse effects of salt on cow liveweight or feed intake. The reduction in urinary N concentration was a dilution effect as daily water intake increased with increasing salt intake (averaged 19, 24 and 34 L/day for 0, 200 or 400 g/day salt treatments respectively). Cow urine volumes increased by 2- and 3-fold when drenched with 200 or 400 g/day salt respectively, compared to the non-salt control treatment. The authors noted an observed increased urination frequency, resulting in similar volume of urine output for each urination event. In a grazing situation this would have resulted in an increased spread of N onto the pasture. If plantain can be used as a diuretic in a grazing situation there is a potential to reduce the concentration of N in urine, N loading at the urine patch whilst increasing the spread of urinary N depositions on grazed pastures. This hypothesis will be explored in the grazing experiments of this thesis.

2.6 Conclusions

- Nitrogen in current pasture species used in New Zealand dairy grazing systems is used inefficiently by grazing animals resulting in high levels on N excreted in urine. High loading of urinary N is the leading cause of nitrate leaching under grazing. Environmental pressures on the dairy industry require systems with reduced nitrate leaching.

- Alternative diverse pastures have been used to reduce urinary N output from grazing dairy cows. Effects of these pastures are likely due to an individual species in the mix than diversity per se.

- Plantain is a suitable forage for use in a grazing situation and may prove a superior species in terms of reducing urinary N concentration due to diuretic properties in the plant that may increase volume and thus spread of urine excreted.

- An increase in the WSC concentration of grasses occurs in the afternoon at the expense of CP and/or NDF. This phenomenon is not well defined for other species. Utilising this diurnal pattern in a grazing situation could further reduce urinary N output due to a reduction in N intake.

Chapter 3

Milk production and urinary N excretion from cows grazing plantain and pasture in early and late lactation

3.1 Introduction

Milk production in New Zealand dairy systems is strongly linked to the quantity and quality of harvested herbage. The predominant pasture used is a binary mixture of perennial ryegrass and white clover. Whilst this pasture is productive under grazing, easy to manage and persists well, there can be issues around nitrogen (N) leaching. There is large discrepancy between the N content of grazed perennial ryegrass-white clover, and the N requirement of the animals. Nitrogen in excess of an animal's requirement is excreted, primarily in the urine (Tamminga 1992). Nitrogen from urine patches is a major contributor to N leaching (Di *et al.* 2007). Reducing the environmental impact of the dairy industry in New Zealand has become a key focus with increasing interest surrounding water quality issues which is affected by nitrogen leaching from pastoral land (Bryant *et al.* 2007; Woodward *et al.* 2012). Regional Councils throughout New Zealand have been developing regulations that place a limit on the amount of nitrate-N leached from agricultural land. Mitigation strategies which reduce urinary-N excretion or divert dietary N away from urine include the use of alternative pasture species. These alternative pasture species vary from perennial ryegrass-white clover in their chemical composition (water-soluble carbohydrate (WSC) and crude protein (CP)), mineral profile and secondary plant compounds.

Previous experiments (Woodward *et al.* 2012; Totty *et al.* 2013; Edwards *et al.* 2015; Bryant *et al.* 2017) achieved lower urinary N from cows by incorporating alternative pastures species including plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*) and additional legumes such as red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*) into pasture mixture with perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). In comparison with standard perennial ryegrass–white clover pastures, these diverse mixtures have shown consistent reductions in urinary N concentration of at least 20% while maintaining or improving milk production (Woodward *et al.* 2012; Totty *et al.* 2013; Bryant *et al.* 2017). However, it is not clear whether the effects are due to an individual species in the mixture rather than the diversity.

Plantain as a pure sward has the potential to be a suitable alternative to perennial ryegrass-white clover pastures for milk production from grazing dairy cows. Previous studies show sheep, beef and deer grazing swards containing plantain had increased liveweight gains compared with animals grazing perennial ryegrass-white clover pastures. These increased liveweight gains have be attributed to; a reduced parasite load (Scales *et al.* 1995), greater dry matter (DM) intake (Golding *et al.* 2008; Kenyon *et al.* 2010) and higher nutritive values (Burke *et al.* 2002; Kemp *et al.* 2010). Further, research using sheep fed plantain or perennial ryegrass diets indicated a positive relationship between plantain feeding and urine volume (O'Connell *et al.* 2016) which may be beneficial to reduce the urinary N concentration by way of dilution.

There is currently little research comparing perennial ryegrass pasture with plantain forage fed as a sole diet. The objective of this study was to assess if plantain as a grazed pasture species can support milk production similar to that achieved with perennial ryegrass-white clover while reducing urinary N losses.

3.2 Materials and methods

3.2.1 Experimental site

Approximately 4.5 ha of pasture split across three 1.5 ha paddocks located at the Lincoln University Research Dairy Farm in Canterbury, New Zealand (43º38’S, 172º27’E) was used for two 10 d grazing experiments (3 d acclimation + 7 day experimental period).

The experiment was located on a Paparua silt loam soil. The site was sown with Arrow ryegrass (20 kg/ha) and white clover (3 kg/ha) 1 April 2014 and rotationally grazed with dairy cows until December 2014. Half of the area was sprayed with round- up on 17 December 2014. Cultivation of the sprayed area took place on 22 and 23 December 2014. This involved rotatory hoe and rolling. Plantain (tonic) was drilled with 75 mm drill rows to the cultivated area at a rate of 10 kg/ha on 23 and 24 December 2014. Poor establishment occurred in paddocks B1 and B2. These were re drilled on 20 January 2015. The area is fully irrigated by centre pivot. Paddocks were rotationally grazed with dairy cows to a constant height and fertilised with urea at a rate of 70 kg N/ha 25 days prior to the experiments. Pasture was irrigated with a centre-pivot irrigator.

3.2.2 Experimental design

Two experiments were conducted at the Lincoln University Research Dairy Farm with the approval of the Lincoln University Animal Ethics Committee in late (AEC 592) and early (AEC 639) lactation in 2015. There were three pasture treatments: (1) a perennial ryegrass-white clover pasture (n =12 cows); (2) plantain (n =12 cows) and (3) an area that was made of 50% perennial ryegrass-white clover and 50% pure plantain by ground area (n=12 cows). Each 1.5 ha paddock was separated into the three forage treatments, using electric fencing. Due to low pasture growth rates in the late lactation study more area was required to meet feed allocation targets so an additional perennial ryegrass & white clover pasture paddock was included for the 50-50 pasture-plantain treatment.

A group of 36 pregnant, lactating Friesian x Jersey dairy cows were blocked into three groups of 12 cows according to milksolids, age, days in milk and liveweight (Table 3.1). Both studies were conducted over a 10-day period with late lactation occurring from 19 to 29 March 2015 and early lactation occurring from 8 to 18 October 2015. This included a 3-day transition period and 7-day experimental period. Before the experiments, all cows grazed perennial ryegrass-white clover pasture together. Cows were milked twice daily at approximately 0600 and 1400 h.

Table 3.1 . Mean milk production, age, days in milk and liveweight of cows (n=36) grazing pasture treatments from 19 to 29 March 2015 (late lactation experiment) and from 8 to 18 October 2015 (early lactation experiment). All values ±SEM

Parameter	Late Lactation Experiment	Early Lactation Experiment
Milk production (kg MS/cow/day)	1.31 ± 0.03	2.48 ± 0.04
Age (years)	5.9 ± 0.4	5.6 ± 0.3
Days in milk	217 ± 3.2	50.9 ± 1.1
Liveweight (kg)	515 ± 8.6	496 ± 8.4

After each morning milking cows were offered a target daily allowance of 18 kg DM/cow/day above 3.5 cm in late lactation and 30 kg DM/cow/day above ground level in early lactation. Daily herbage allocation during the experiment was based on a national calibration equation for perennial ryegrass-white clover (kg DM/ha = 140 × Rising Plate Meter (RPM) reading +150) and previously derived calibration equations between herbage mass and pasture height for plantain pastures (kg DM/ha = 94 × RPM reading + 455) (Haultain *et al.* 2014). For the 50-50 pasture-plantain treatment pre-grazing plate meter readings were taken in both the pasture and plantain areas. The sum of 0.5 × pasture herbage mass and 0.5 × plantain herbage mass was used to determine the area to be grazed as spatially separated pasture and plantain. Daily allocated areas were controlled by temporary electric fencing. Back fencing was used to prevent grazing of residual regrowth. Cows had *ad lib* access to water through a portable trough.

Plate 3.1: Cows on the 50-50 perennial ryegrass-white clover-plantain treatment grazing spatially separated perennial ryegrass-white clover and plantain pastures in late lactation 2015 at the Lincoln University Research Dairy Farm

3.2.3 Herbage measurements

At least 50 compressed pasture height measurements were recorded daily pre- and post-grazing using a calibrated rising plate meter (RPM; Jenquip, Filip's EC 09, Electronic Folding plate meter). The pre-grazing measurements were recorded in the area estimated to be allocated in the next forage allocation. Calibration measurements were collected from pastures every second day by cutting two 0.2 m^2 quadrats to ground level in late lactation and five in early lactation before and after grazing during each experiment. Two RPM measurements were recorded in each quadrat prior to harvesting herbage. Collected herbage samples were weighed fresh and a subsample taken to determine botanical composition of pastures. All botanical components and bulk samples were oven dried at 65^{o}C for 48 h and total dry weight and DM% determined. Linear and curvilinear relationships between herbage mass and pasture height were compared and best-fit equations (greatest r^2) were fitted to the data. The calibration equations for each pasture type are shown in Table 3.2.

Table 3.2 Pre and post grazing combine calibration equations for early and late lactation experiments. R^2 represents the relationship to a fitted linear regression model

Experiment	Pasture type	Equation	R^2
Early lactation	Ryegrass-white clover	145 × height + 3.92	0.85
	Plantain	73 × height + 248	0.89
Late lactation	Ryegrass-white clover	120 × height + 312	0.63
	Plantain	114 × height + 470	0.71

Using equations derived from experimental data sets and grazing areas, the actual daily herbage allocation was calculated as 21.4 ± 2.4 kg/DM/cow for pasture, 19.1 ± 0.94 kg/DM/cow for plantain and 22.2 ± 0.9 kg/DM/cow for 50-50 pasture-plantain in late lactation. In early lactation actual daily herbage allocation was calculated as 32.8 ± 1.3 kg/DM/cow for pasture, 31.3 ± 0.65 kg/DM/cow for plantain and 31.6 ± 0.37 kg/DM/cow for 50-50 pasture-plantain. Apparent group DM intake of cows was calculated from herbage disappearance between pre- and post-grazing calibrated RPM measurements and areas allocated using the equation:

$$DMI = \frac{\left(\frac{m^2 allocated}{10{,}000}\right) \times pre\ DM \left(\frac{kg}{ha}\right) - post\ DM \left(\frac{kg}{ha}\right)}{Number\ of\ cows}$$

Subsampled herbage was sorted into sown species, weeds and dead material. Sorted material and a bulk sample of the pasture was oven dried at 65°C for 48 h to determine botanical composition of pastures. Bulk oven-dried samples were ground through a 1 mm sieve and scanned by near infra-red spectrophotometer (NIRS, NIRSystems 5000, Foss, Maryland, USA) to determine crude protein (CP), digestible organic matter in the dry matter (DOMD), water soluble carbohydrate (WSC, including soluble starch), acid detergent fibre (ADF) and neutral-detergent fibre (NDF). Calibration equations for NIRS were derived from perennial ryegrass, clover and plantain herbage. Ground samples were then subsampled and analysed for mineral composition by inductively coupled plasma atomic emission spectroscopy and secondary plant compounds (catalpol, aucubin and acteoside) analysed for plantain only using high performance liquid chromatography by Massey University analytical laboratory following procedures of Tamura *et al.* (2002).

Metabolisable energy (ME) was calculated using the equation (CSIRO 2007):

$$MJME\ /\ kg\ DM = 0.16\ \times DOMD$$

Nutrient intake per cow was calculated from the difference between pre and post grazed herbage nutrient available using the equation:

$$N_{intake} = \frac{(pre\ DM\ \ \times pre\ N) - (post\ DM\ \ \times post\ N)}{number\ of\ cows}$$

Where pre and post DM are the mass of DM/ha allocated and pre and post N is the concentration (%) of the nutrient in the pre and post grazed herbage respectively.

3.2.4 Animal measurements

3.2.5 Milk measurements

Milk yield was measured daily for individual cows with an automated system (DeLavel Alpro Herd management system, DeLavel, Tumba Sweden). Milk samples from all cows were collected on days 4, 6, 8 and 10 from the afternoon and following morning milking to determine milk composition and milk urea nitrogen (MUN). Fat, protein and lactose composition in milk were analysed by Livestock Improvement Corporation Ltd (Christchurch, New Zealand) by MilkoScan (Foss Electric, Hillerod, Denmark). Samples for MUN were centrifuged at 4,000 × g for 10 min at room temperature and refrigerated for 10 min to allow the fat to solidify on the top and be removed. Skim milk was pipetted into a clean micro-centrifuge tube, and frozen for later analysis of thawed samples using automated Modular P analyser (Roche/Hitachi).

3.2.6 Urine and fecal measurements

Urine compositon measurements

Immediately after afternoon and the following morning milk on days 5, 7 and 9 of the experiments, cows were herded into the veterinary yards for urine and faecal sample collection. Spot urine samples were collected mid-stream by vulval stimulation. In the late lactation experiment urine samples were immediately acidified to below a pH of 4.0 with concentrated sulphuric acid to prevent volatilization and stored at -20°C. In early lactation a pH reading of the urine was taken immediately after sample collection using a pH reader then acidified with sulphuric acid before storing at -20^{o}C. Urine N content of thawed samples was determined by autoanalyser (Roche Cobas Mira Plus CC, Minnesota, USA). Urea content of acidified urine was analysed using a commercial enzymatic kinetic technique (Randox, Crumlin, Co. Antrim, UK). Creatinine content of acidified urine was determined calorimetrically using a commercial kit from Randox laboratories (Crumlin, Co. Antrim, UK). Purine derivative (PD) concentration in the urine was using HPLC (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) as previously described by Czauderna *et al.* (2000) with modifications by George *et al.* (2006).

Faecal compostion measurements

Faecal samples were obtained from voluntary events in the yards or by rectal stimulation and stored at -20°C. Thawed faecal samples were sub sampled, with one sample being weighed and then dried in an over at 65^{o}C for 48 h and reweighed to ascertain dry matter. A second subsample was freeze dried and ground through a 1 mm sieve. Faecal N content was determined by combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH. Hanau, Germany).

Urine volume and N output measurements

A urine meter harness designed by Ravera *et al.* (2015) was worn by all cows for up to 24 hours between days 5 and 10 for both experiments. The urine harness consisted of a thick reinforced rubber glove which was superglued (Henkel's Loctite Power Flex Gel) to fit into a pointed oval shaped hole in a piece of vinyl covered upholstery fabric (16.5 cm x 11.5 cm). This formed the opening of the harness to be attached to the cow. All but one finger of the rubber glove were excluded by knotting tightly with string. The remaining finger was attached to a pipe connector piece using a cable tie – this pipe connector was used to attach a flow meter sensor.

The fabric of the harness was attached to the rear of the cow using super glue (Henkel's Loctite Power Flex Gel) approved for use on animals. Strapping tape was used to provide additional adhesion of the fabric to the cow and to prevent pulling of the edges. Covers with pockets on either side of the shoulder were fitted to cows. A Velcro strap running the length of the cover was used to hold a wire running from the flow meter sensor to a radio logger contained in one of the pockets. Stones were placed in the second pocket as a counter weight. On occasions the urine meter would fail through becoming unstuck, wire breakage or sensor failure. This left 20 cows in late lactation from which data was used and 22 cows in early lactation.

Plate 3.2: Cows wearing the urine harness

Urinary N output was estimated using three methods. (1) urine volumes measured using the urine harness were multiplied by overall treatment urine N concentration averages, (2) creatinine-based equation (Pacheco et al. 2007):

$$urinary\ N\ (g\ /d) = \left(21.9\ (mg/kg) \times LW\ (KG) \times \left(\frac{1}{urinary\ creatinine\ (mg/kg)}\right)\right) \times urine\ N\ (g/kg)\ )$$

and (3) MUN based equation (Jonker *et al.* 1998):

$$urinary\ N\ (g/d) = 0.026\ \times LW\ (kg) \times milk\ urea\ N\ (mg/dL)$$

3.2.7 Estimation of microbial N supply

Effect of treatment on rumen microbial activity was calculated from the ratio of purine derivatives (PD) to creatinine in spot samples of urine (Chen 1989; Verbic *et al.* 1990). The resulting PD index is a relative measure of microbial protein synthesis:

$$\text{PD index} = \frac{total\ PD\ (mmol/L)}{creatinine\ (mmol/L)} \times BW^{0.75}$$

Where

$$total\ PD\ = allantoin\ (mmol/L)\ +\ uric\ acid\ (mmol/L).$$

3.2.8 Statistical analysis

Treatment means for milk, urine and faeces were determined using data from individual measurements from animal samples over sampling days. Intake and herbage measurements were estimated as means for the treatment group as animals grazed together in their treatment groups. The effect of pasture type on milk, urine and faecal measurements was analysed for variance using GenStat 15.1 (VSN International LTD. 2012), with cows as random effect and pasture type as fixed effect using a one-way ANOVA. Herbage measurements were also analysed by one-way ANOVA using Genstat with sampling day as the replicate. Results were declared to be significant at P<0.05. Means were separated using Fisher's protected least significance difference test.

3.3 Results

3.3.1 Herbage

Herbage characteristics and composition of plantain and perennial ryegrass-white clover pastures are shown in Table 3.3 and Table 3.4. In the late lactation experiment, pre-grazing mass of the pasture portion of the 50-50 pasture plantain was 700 kg DM/ha greater (P<0.001) than the herbage mass for any other treatment (Table 3.3). Dry matter content of plantain was nearly half (P<0.001) that of perennial ryegrass-white clover in late lactation and about 20% lower (P=0.008) in early lactation. Organic matter content was lower (P<0.001) in plantain than in perennial ryegrass-white clover in late lactation only.

The post-grazing mass of plantain was lower (P<0.001) than that of pasture in both late and early lactation (Table 3.3; Table 3.4). When plantain was included in the diet the apparent DM intake increased by about 20% (P<0.001, Table 3.6). In early lactation apparent DM intake was greater (P=0.017) for the plantain treatment only. The ME and CP were similar across pasture treatments for both seasons (P>0.05, Table 3.2). Similarly, apparent N intake and ME intake did not differ across treatments.

The mineral composition of pasture and plantain is shown in Table 3.5. The mineral concentration was similar for pasture and plantain except for sodium and calcium. Sodium concentration was two times greater (P<0.001) in plantain than pasture in late lactation only. Calcium concentration of the herbage was about three times greater (P<0.001) in plantain than pasture for late and early lactation. Of the secondary plant compounds measured acteoside had the highest concentration in plantain (Table 3.5). Catalpol was present in very low concentrations (<0.09 %). The concentration of all secondary compounds in plantain tended to be higher in late lactation.

Total average water intake was similar for all treatments (150 L/cow/day) and more than two thirds of total water was ingested in feed. As plantain intake increased, water intake from feed increased and drinking water declined (Table 3.6).

Table 3.3 Mean herbage characteristics and chemical composition of pasture, plantain or 50-50 pasture-plantain sampled to ground level for late lactation 2015. LSD = least significant difference ($\alpha = 0.05$). Means followed by different letters denote that values are significantly different at the 5% level

	Pasture	50-50 pasture-plantain		Plantain	LSD	P Value
		Pasture	Plantain			
Pre-grazing						
Herbage mass (kg DM/ha)	3251 ^{a}	3935 ^{b}	3005^{a}	3224^{a}	301	<0.001
OM (%)	89.9^{a}	90.2^{a}	85.1^{b}	85.0^{a}	1.67	<0.001
N (%)	3.74	3.37	3.68	3.57	0.386	0.215
ME (MJ ME/kg DM)	11.1	10.9	11.3	11.0	0.388	0.240
ADF (%)	276^{a}	297^{a}	212^{b}	228^{b}	27.0	<0.001
WSC (g/kg DM)	79.3^{bc}	55.6^{c}	123^{a}	103^{ab}	39.4	0.015
NDF (g/kg DM)	419^{b}	504^{a}	277^{c}	305^{c}	62.1	<0.001
CP (g/kg DM)	234	211	230	223	24.1	0.215
Plantain (%)	0^{b}	0^{b}	89.7^{a}	89.6^{a}	4.530	<0.001
Grass (%)	48.4^{b}	58.8^{a}	0^{b}	0^{b}	8.953	<0.001
Legume (%)	25.7^{a}	15.1^{b}	0^{c}	0^{c}	0.066	<0.001
Weed (%)	3.80	6.98	3.72	6.10	5.542	0.280
Dead (%)	22.1^{a}	25.4^{a}	6.62^{b}	4.31^{b}	7.831	<0.001
DM (%)	17.2^{a}	16.8^{a}	10.7^{b}	9.84^{b}	2.207	<0.001
Post-grazing						
Herbage mass (kg DM/ha)	1198^{a}	1024^{b}	638^{c}	536^{d}	58.2	<0.001

Table 3.4 Mean herbage characteristics and chemical composition of pasture, plantain or 50-50 pasture-plantain sampled to ground level for early lactation 2015. LSD = least significant difference (α = 0.05). Means followed by different letters denote that values are significantly different at the 5% level

	Pasture	50-50 pasture-plantain		Plantain	LSD	P Value
		Pasture	Plantain			
Pre-grazing						
Herbage mass (kg DM/ha)	3705	3886	3611	3800	418.6	0.571
OM (%)	90.3	90.4	69.7	78.4	20.24	0.126
N (%)	2.98	2.69	3.25	3.12	0.455	0.091
ME (MJ ME/kg DM)	11.7	11.7	12.1	11.8	0.404	0.197
ADF (g/kg DM)	265^{ab}	266^{a}	230^{bc}	247^{c}	19.2	0.003
WSC (g/kg DM)	141^{a}	153^{a}	60.2^{b}	60.7^{b}	35.13	<0.001
NDF (g/kg DM)	460_{a}	469^{a}	328^{b}	340^{b}	43.4	<0.001
CP (g/kg DM)	186	168	203	195	28.4	0.091
Plantain (%)	0^{c}	0.6^{b}	66.7^{a}	67.8^{a}	12.49	<0.001
Grass (%)	74.7^{a}	74.3^{a}	0^{b}	0^{b}	15.47	<0.001
Legume (%)	7.28^{a}	7.90^{a}	0^{b}	0^{b}	0.958	<0.001
Weed (%)	3.90^{b}	1.66^{b}	25.3^{a}	20.8^{a}	14.78	<0.001
Dead (%)	14.2^{a}	15.5^{a}	7.98^{b}	11.4^{ab}	5.44	0.039
DM (%)	17.2^{a}	18.0^{a}	14.8^{b}	14.4^{b}	2.26	0.008
Post-grazing						
Herbage mass (kg DM/ha)	1580^{ab}	1664^{a}	1437^{bc}	1281^{c}	192.4	0.002

Table 3.5 Mean herbage mineral composition (% DM) bioactive glycoside (mg/g dry DM) of pasture, plantain or 50-50 pasture-plantain sampled to ground level. LSD = least significant difference (α = 0.05). Means followed by different letters denote that values are significantly different at the 5% level

	Pasture	50-50 pasture-plantain		Plantain	LSD	P Value
		Pasture	Plantain			
Late lactation 2015						
Sodium	0.380^{b}	0.420^{b}	0.965^{a}	0.985^{a}	0.3486	<0.001
Magnesium	0.247^{a}	0.250^{a}	0.197^{ab}	0.183^{b}	0.0541	0.045
Calcium	0.807^{b}	0.583^{b}	2.26^{a}	2.13^{a}	0.260	<0.001
Chloride	1.58	1.52	2.07	2.04	1.211	0.619
Sulphur	0.270	0.260	0.367	0.327	0.1124	0.177
Potassium	3.47	3.40	3.53	3.13	1.231	0.883
Phosphorus	0.363	0.383	0.410	0.383	0.0711	0.541
DCAD	44	461	513	448	263.4	0.919
K/Na Ratio	9.33	10.33	3.67	3.33	7.61	0.134
Catalpol			0.063	0.088	0.1136	0.710
Aucubin			5.67	4.66	2.861	0.382
Acteoside			9.29	11.3	6.73	0.445
Early lactation 2015						
Sodium	0.691	0.894	1.07	1.10	0.5436	0.342
Magnesium	0.193	0.183	0.183	0.190	0.0421	0.927
Calcium	0.607^{b}	0.787^{b}	1.77^{a}	2.12^{a}	0.3814	<0.001
Chloride	1.16	1.83	2.64	2.95	2.050	0.254
Sulphur	0.280	0.313	0.400	0.367	0.1124	0.142
Potassium	2.60	2.67	3.20	3.03	1.517	0.767
Phosphorus	0.420	0.343	0.400	0.367	0.0886	0.269
DCAD	464	370	297	192	367.6	0.426
K/Na Ratio	4.33	3.00	3.00	2.67	3.216	0.655
Catalpol			0.033	0.032	0.0373	0.953
Aucubin			2.51	1.88	3.880	0.676
Acteoside			7.60	4.93	9.46	0.478

Table 3.6 Apparent intake of dairy cows grazing pasture, plantain or 50-50 pasture-plantain in late and early lactation and water intake in early lactation. LSD = least significant difference ($\alpha = 0.05$). Means followed by different letters denote that values are significantly different at the 5% level

	Pasture	50-50 pasture-plantain	Plantain	LSD	P Value
Late lactation 2015					
Kg DM/cow/d	13.7^{b}	16.9^{a}	15.9^{a}	1.45	<0.001
MJ ME/cow/d	169	197	185	29.2	0.155
N intake/cow/d	619	518	594	182	0.459
Early lactation 2015					
Kg DM/cow/d	18.5^{a}	18.5^{a}	20.7^{b}	1.64	0.017
MJ ME/cow/d	234	245	275	44.1	0.061
N intake/cow/d	652	629	669	148.5	0.829
Water intake (L/cow/d)					
From trough	40.3^{a}	22.8^{b}	10.0^{c}	7.32	<0.001
From herbage	109	115	151	50.9	0.167
Total	147	140	163	51.7	0.590

3.3.2 Milk production and composition

Milk volume (L) and milksolids (kg) yield was higher ($P<0.05$) for pure plantain than perennial ryegrass pasture in late lactation though there were no treatment differences in early lactation (Table 3.7). In both early and late lactation, perennial ryegrass and plantain (50-50) altered milk composition. In late lactation milk fat percent was lower and lactose percent was higher when cows grazed 50-50 pasture-plantain compared with perennial ryegrass pasture alone (Table 3.7). In early lactation milk protein percent was lower for the 50-50 treatment than the perennial ryegrass pasture treatment. Milk urea N was lowest ($P<0.005$) for plantain in both late and early lactation.

3.3.3 Urine and faecal N

The concentration of urea and N in urine was lowest ($P<0.001$) for plantain, intermediate for 50-50 pasture-plantain and highest in pasture in both late and early lactation (Table 3.8). In late lactation the concentration of NH_3 in the urine was lowest for plantain. There was no difference in NH_3 in the urine among treatments in early lactation. Estimated urinary N output was lowest for plantain for all methods used in both late lactation and early lactation (Table 3.8). The concentration of N in the faeces was similar among treatments in late lactation.

Table 3.7 Mean milk yield and composition (n=12) of dairy cows grazing perennial ryegrass-white clover pasture, plantain or 50-50 pasture-plantain. LSD = least significant difference (α = 0.05). Means followed by different letters denote that values are significantly different at the 5% level

	Pasture	50-50 pasture-plantain	Plantain	LSD	P value
Late lactation (autumn 2015)					
Milk volume (L)	14.3^{a}	16.3^{b}	16.4^{b}	0.76	<0.001
Milk solids (kg/d)	1.50^{a}	1.60^{ab}	1.67^{b}	0.08	0.012
Milk protein (%)	4.28	4.29	4.34	0.09	0.512
Milk protein (kg)	0.62^{a}	0.70^{b}	0.72^{b}	0.03	<0.001
Milk fat (%)	6.16^{a}	5.52^{b}	5.80^{ab}	0.23	<0.001
Milk fat (kg)	0.88	0.90	0.95	0.05	0.142
Lactose (%)	4.95^{b}	5.07^{a}	5.05^{a}	0.04	<0.001
Urea N (mmol/L)	11.2^{a}	10.9^{a}	9.96^{b}	0.54	0.005
Early lactation (spring 2015)					
Milk volume (L)	27.1	27.9	26.9	1.14	0.169
Milk solids (kg/d)	2.42	2.43	2.39	0.010	0.772
Milk protein (%)	3.75^{a}	3.67^{b}	3.72^{ab}	0.060	<0.001
Milk protein (kg)	1.02	1.03	1.00	0.037	0.596
Milk fat (%)	5.48	5.32	5.38	0.230	0.394
Milk fat (kg)	1.40	1.41	1.39	0.075	0.886
Lactose (%)	5.18	5.15	5.16	0.043	0.461
Urea N (mmol/L)	8.23^{a}	6.07^{b}	5.15^{c}	0.602	0.001

Table 3.8 Mean urine and faecal N characteristics (n=12) and urine volume and urination frequency of dairy cows grazing perennial ryegrass-white clover pasture, plantain or 50-50 pasture-plantain. LSD = least significant difference (α = 0.05). Means followed by different letters denote that values are significantly different at the 5% level.

	Pasture	50-50 pasture-plantain	Plantain	LSD	P value
Late lactation (autumn 2015)					
Urine					
NH_3 (mmol/L)	1.98^{a}	1.35^{b}	0.97^{c}	0.30	<0.001
Urea (mmol/L)	144.3^{a}	94.8^{b}	61.5^{c}	15.8	<0.001
N concentration (g N/L)	5.4^{a}	3.6^{b}	2.4^{c}	0.06	<0.001
Creatinine (mmol/L)	1.67^{a}	1.27^{b}	0.88^{c}	0.24	<0.001
Total volume/day (L/d)[1]	46.5 (3)	59.1 (3)	73.8 (3)	23.65	0.079
Urination frequency (events/d)[1]	18.0 (3)	18.3 (3)	20.0 (3)	6.66	0.745
Average volume/urination (L)[2]	3.23 (95)	2.87 (88)	3.34 (86)	0.552	0.228
N output/d	251	213	177		
N output/d - creat	337^{a}	304^{b}	275^{b}	32.1	<0.001
N output/d - MUN	404^{a}	375^{b}	346^{c}	25.8	<0.001
Faeces					
N (%)	3.43	3.33	3.45	0.11	0.070
DM (%)	10.9^{a}	12.6^{b}	15.7^{c}	0.86	<0.001
Early lactation (spring 2015)					
Urine					
NH_3 (mmol/L)	1.17	1.30	1.69	0.619	0.239
Urea (mmol/L)	113.8^{a}	71.8^{b}	44.5^{c}	13.33	<0.001
N concentration (g N/L)	4.7^{a}	3.4^{b}	2.2^{c}	0.046	<0.001
Creatinine (mmol/L)	1.66^{a}	1.49^{ab}	1.26^{b}	0.238	0.004
pH	8.09^{a}	7.92^{b}	7.67^{c}	0.127	<0.001
Total volume/day (L/d)[1]	43.6 (2)	33.6 (2)	54.1 (3)	33.31	0.331
Urination frequency (events/d)[1]	15.5 (2)	11.5 (2)	18.3 (3)	7.59	0.141
Average volume/urination (L)[2]	2.75 (67)	2.82 (51)	3.09 (66)	0.491	0.325
N output (g N/d)	205	114	119		
N output/d - creat	298^{a}	232^{b}	184^{c}	24.19	<0.001
N output/d - MUN	304^{a}	218^{b}	189^{c}	17.7	<0.001
Faeces					
N (%)	3.97^{a}	3.85^{a}	3.60^{b}	0.098	<0.001
DM (%)	11.6	9.90	11.0	3.306	0.581

[1] Values in parenthesis are the total number of cows on which urine meter data was recorded. [2] Values in parenthesis represent the total number of measurements from the urine meter.

3.3.4 Microbial protein supply

Urinary PD concentrations and calculations associated with estimates of microbial N supply are presented in Table 3.9. The concentration of purine derivatives allantoin, uric acid and hippuric acid were lowest ($P<0.001$) for plantain in both late lactation and early lactation. All purine derivative concentrations tended to be higher in early lactation than late lactation for all treatments.

Table 3.9: Mean urine purine derivative (PD) concentrations (mmol/L) of dairy cows (n=12) grazing perennial ryegrass-white clover pasture, plantain or 50-50 pasture-plantain. LSD = least significant difference ($\alpha = 0.05$). Means followed by different letters denote that values are significantly different at the 5% level.

	Pasture	50-50 pasture-plantain	Plantain	LSD	P value
Late lactation (autumn 2015)					
Allantoin	5.78^a	4.91^b	3.05^c	0.836	<0.001
Uric acid	0.410^a	0.364^a	0.264^b	0.068	<0.001
Hippuric acid	7.19^a	4.37^b	2.58^c	1.212	<0.001
Xanthine	0.002	0.005	0.002	0.063	0.386
Hypoxanthine	0.001^a	0.039^b	0.000^a	0.002	0.006
PD index[1]	409^b	455^a	401^b	37.0	0.009
Early lactation (spring 2015)					
Allantoin	9.71^a	8.81^a	6.63^b	1.205	<0.001
Uric acid	0.769^a	0.695^a	0.490^b	0.1052	<0.001
Hippuric acid	13.9^a	11.1^b	5.6^c	2.13	<0.001
Xanthine	0.000	0.001	0.001	0.0010	0.097
Hypoxanthine	0.001^a	0.003^b	0.003^b	0.0017	0.034
PD index	720^a	682^a	617^b	54.5	<0.001

[1] purine derivative index, PD index = $\frac{total\ PD\ (\frac{mmol}{L})}{creatinine\ \left(\frac{mmol}{L}\right)} \times BW^{0.75}$

3.4 Discussion

The hypothesis of the present study was that the use of plantain as a grazed pasture species would be suitable to achieve similar milk production to that achieved by feeding perennial ryegrass-white clover, while reducing urinary N losses. The results from this study support this hypothesis. In these experiments pure plantain swards as 100% or 50% of a cow's forage allowance reduced the concentration of N in the urine and estimated N excretion per day while maintaining or improving milk production.

3.4.1 Milk production

Milksolids produced per cow in late lactation were greatest for cows grazing plantain (1.67 kg) compared to pasture (1.50 kg) with 50-50 pasture-plantain intermediate (1.60 kg). This was largely due to an increase in milk volume as milk protein percentage was unaffected by pasture treatment and fat percentage tended to be lower where plantain was included in the diet. An overall increase in milksolids and reduction in milk fat was also observed by Totty *et al.* (2013) when plantain was included in a mixed pasture. A possible explanation for the increase in milk volume was the increase in apparent DMI for cows on plantain in late lactation. This occurred because plantain was grazed to a lower post grazing herbage mass compared with pasture despite similar daily allocations of herbage. However, it is noteworthy that cows grazing 50-50 pasture-plantain had the highest apparent DMI in late lactation and this did not translate to increased milk production. In early lactation milk production was not different between pasture types, despite some differences in apparent DMI. In a related study, Edwards *et al.* (2015) reported similar differences in DMI between simple and diverse pastures (15.3±2.1 vs. 16.2±1.4kg DM/cow/day respectively) that did not change the milk yield and composition.

In late lactation cows grazing plantain and 50-50 plantain-pasture showed an increase in lactose concentration in the milk. This may indicate changes in rumen fermentation and variation in the supply of volatile fatty acids. Lactose is influenced by glucose supply and production of gluconeogenic precursors such as propionate (Bauman *et al.* 1980). Diets high in starch or water soluble carbohydrate increase propionate and thereby glucose production. Plantain in autumn (late lactation) had a WSC concentration twice as great as perennial ryegrass-white clover. In early lactation there was no difference in lactose concentration which coincided with a lower WSC concentration of plantain compared with late lactation WSC concentrations.

The proportion of milk fat in the milk was lower for cows grazing plantain than perennial ryegrass-white clover in late lactation. This has been inconsistently seen previously when plantain has been included in a mix species pasture. Bryant *et al.* (2017) and Edwards *et al.* (2015) found no difference in the concentration of milk fat between cows grazing perennial ryegrass-white clover pastures and those on diverse pastures which contained about 15% plantain. However similar to the current experiment, Totty *et al.* (2013) found milk fat was reduced when plantain was included in a mix with chicory, lotus, high-sugar ryegrass, and white clover. The results of previous experiments and the current, show inconsistencies on the effects of plantain on milk composition and further research may be necessary.

The reduced milk fat proportion in the current experiment and seen by Totty *et al.* (2013) may be attributed to the lower fibre content of plantain. Sutton *et al.* (1980) reported that as fibre intake declines, milk fat percentage falls proportionately; however, milk fat yields may increase if overall DM intakes are increased. In this experiment the NDF of plantain was lower than that of perennial ryegrass-white clover. This may help to explain the decline in milk fat percent for cows grazing plantain.

While results from this study and others reported have not shown a consistent improvement in livestock production when plantain is included in the diet, no studies have reported a decrease in production associated with feeding plantain. It therefore can be concluded that plantain is a suitable, graze-able, feed option to include with perennial ryegrass-white clover or include as a monoculture for maintaining or improving milk production. Due to increased DM production of plantain compared with perennial ryegrass (Nobilly *et al.* 2012; Martin *et al.* 2017) plantain may be particularly beneficial in regions and times where perennial ryegrass quality and supply is limited by climatic factors.

3.4.2 N partitioning

Previous short duration experiments have shown that when plantain is included in mixtures with perennial ryegrass, white clover and chicory, urinary N concentration of dairy cows was reduced by at least 20% compared with perennial ryegrass-white clover pastures (Woodward *et al.* 2012; Totty *et al.* 2013). In the current experiment, when plantain was included in the diet at 50% or more of the allocation, urinary N concentration was more than 30% lower, in both early and late lactation, compared with perennial ryegrass-white clover pasture only. The MUN concentration, an indicator of surplus dietary N (Cosgrove *et al.* 2014), was also lower for plantain-fed cows than for pasture-fed cows. Previous studies have shown the excretion of N in urine to be linearly related to N intake (Tas *et al.* 2006b; Higgs *et al.* 2012). The current experiment indicated that factors other than N intake may have been a driver of lower urinary N concentration, as there is no clear relationship with N intake across the dietary treatments.

There is some evidence from *in vitro* studies that aucubin and acteoside may reduce rumen ammonia formation (Navarrete *et al.* 2016). There was no difference in faecal nitrogen concentration, suggesting this was not a route of increased excretion. Similar results have been seen by Judson *et al.* (2016) where urinary N concentration was reduced by about 30% for heifers supplemented with plantain silage despite having similar N intakes to those with no plantain in their diet. There may have been some differences in energy and protein supply to the rumen indicated by the lower PD index for cows on plantain (Chen 1989).

The urinary N concentration can be influenced by total urine volume. The total volume of urine excreted per day by animals grazing plantain (74 L) in late lactation measured by the urine harness was 57% greater than the volume of urine excreted by cows grazing pasture (47 L). Further, creatinine, a marker of urine volume (Chizzotti *et al.* 2008; Waldrip *et al.* 2013) was lower for cows on plantain diets in both experiments which suggested a higher urinary output in both experiments for diets containing plantain. This may have been a factor of higher mineral content, secondary plant compounds or increased water intake due to the lower DM% of plantain. Plantain in both late and early lactation had a greater calcium and sodium mineral content than pasture. The concentration of sodium, a known diuretic (Ledgard *et al.* 2015), was almost twice as much in plantain compared with pasture for late lactation only. This coincided with an observed increase in urine volume indicating that sodium may have been a factor. This increase in urine volume was supported by recent data which strongly suggests a sustained diuretic effect when plantain was fed to sheep (O'Connell *et al.* 2016). Though not measured, O'Connell *et al.* (2016) suggested that diuresis may have been due to bioactive compounds known to exist in the leaves of plantain.

Bioactive compounds in plantain have been studied in early medicinal work (Nishibi *et al.* 1995). The major compounds in plantain include iridoid glucosides catalpol and aucubin and phenylethanoid glucoside acteoside. Although catalpol is a known diuretic (Tamura *et al.* 2002) the availability of the compound in this experiment is unlikely to explain the higher urine volume output for cows grazing plantain as its concentration was low (<0.08 g/kg DM). The aucubin and acteoside were within range of findings from Navarrete *et al.* (2016) (aucubin 1.78 to 3.80 g/kg DM and acteoside 0.5 to 41.7 g/kg DM). These compounds may help to explain the greater urine output seen for animals grazing plantain pastures. The concentration of aucubin and acteoside were greatest in late lactation which coincided with higher urine outputs. Aucubin is known to stimulate the excretion of uric acid from the kidneys (Kato 1946). However, in the current experiment, the concentration of uric acid in the urine was lower for cows grazing pure plantain compared with those on pasture.

Despite greater total volume output, N output was not increased for cows grazing plantain. The lower urinary N concentration from cows grazing plantain was able to offset the increase in calculated or measured urine volume, resulting in a reduction in total N output when plantain was included in the diet. Using average urination size and assuming a patch size of 0.2 m^2, the urine N loading from cows on perennial ryegrass-white clover pasture was about 700 kg N/ha in late lactation and 670 kg N/ha in early lactation. With the same assumptions a urine patch from cows grazing plantain would have a N loading of about 450 kg N/ha in late lactation and 320 kg N/ha in early lactation. Application rates above 500 kg N/ha will likely increase leaching and nitrous oxide emission potential (Ledgard *et al.* 2007; Groenigen *et al.* 2010). This shows the potential of plantain to reduce N losses from grazing dairy systems.

3.5 Conclusions

- These results demonstrate a role for the use of plantain as a mitigation strategy to reduce the environmental impact of dairy farming.
- By providing plantain as a monoculture or with perennial ryegrass-white clover pastures to cows, milksolids production was increased or maintained and urine N concentration was reduced.
- The decline in urine N concentration and urinary N output for cows grazing plantain at 100 or 50% of their diet may decrease urine patch N loading, thereby reducing the risk of nitrate leaching for dairy grazing systems.

Chapter 4

Diurnal characterisation of the nutritive composition of six forages at high and low N

4.1 Introduction

Livestock production in New Zealand grazing systems is directly linked to the quantity and quality of harvested herbage. In dairy systems the predominant pasture used is a binary mixture of perennial ryegrass and white clover. However, the composition of nutrients available from perennial ryegrass based pastures to grazing animals varies widely due to seasonal and diurnal changes. A ryegrass based pasture can have low crude protein (CP) and high fibre concentrations in summer, which may restrict feed intake, so that animal nutrient requirements are often not met (Waghorn 2002). At other times of the year, a ryegrass based pastures can have a high concentration and solubility of CP (Bryant *et al.* 2012) resulting in a low utilization efficiency and a large proportion of dietary N being excreted in the urine (Tamminga 1992). Urinary N losses are an environmental concern and mitigation strategies are sought to meet new regulations.

A positive relationship between CP intake and urinary N losses has been well established (Tas *et al.* 2006b). Therefore a logical pathway to reduce urinary N losses is to provide animals with a forage that has a lower N concentration, thereby reducing total N intake (provided DM intake remains the same). This would require a characterisation of potential forage species which are suitable for use in an outdoor grazing system. Multi-species pastures which include herbs (plantain and chicory) have been identified as a means of reducing urinary N concentration compared with a ryegrass-white clover pasture, while maintaining or improving milk production (Woodward *et al.* 2012; Totty *et al.* 2013; Edwards *et al.* 2015; Bryant *et al.* 2017). Further, in Chapter 3 when plantain was fed as a monoculture alone or in conjunction with perennial ryegrass-white clover, urinary N concentration was reduced by over 30%. However, the mechanisms contributing to this effect are unclear.

Another management tool that could be utilised as a mitigation strategy, is to allocate pasture in the afternoon to reduce the proportion of N in the herbage, and thus the supply to the animal (Moorby *et al.* 2006; Bryant *et al.* 2014). Ryegrass shows a diurnal change in water soluble carbohydrate (WSC) concentration, increasing from morning to night (Miller *et al.* 2001). This increase in WSC is offset by a decline in CP and fibre. In a previous experiment when an elevated WSC concentration was achieved in high sugar ryegrass by harvesting in the afternoon, urinary N output was reduced by 28.7 g/cow/d compared with a non-selected ryegrass harvested in the morning (Miller *et al.* 2001). Forage species which show large diurnal increases in WSC at the expense of CP may be valuable to supply nutrients to the animal in more efficient way.

While diurnal changes in the chemical composition of ryegrass have been well studied less information is available for alternative herb and legume species (e.g. plantain, chicory, red and white clover). The purpose of this study was to compare the effect of N inputs and harvest time on herbage nutritive value of conventional and alternative pasture forages.

4.2 Materials and Methods

4.2.1 Experimental site and design

The experiment was conducted from 8 October 2014 to 18 May 2015 at the Lincoln University Research Dairy Farm, Canterbury New Zealand (43^{o}64'S, 172^{o}46'E). A split-split plot design was used with 3 replicates. Treatments were forage type (6 species), N rate (two levels) and harvest time (am and pm). The six forage types were; diploid ryegrass, Italian ryegrass, plantain, chicory, white clover and red clover (Table 4.1). N fertiliser was applied following each defoliation as calcium ammonium nitrate (27 : 0 : 0 : 0; N : P : K : S), with the total annual N application rate split evenly throughout the year. Before the experiment began, the planned N fertiliser treatments were 200 and 500 kg N/ha/year. It was estimated that nine harvests for legumes and ten harvests for grasses and herbs would occur. However, due to best practice methods, legumes were harvested seven times and grasses and herbs harvested nine times. This resulted in lower levels of N applied than proposed and differences in rates of N fertiliser applied between legumes and grasses and herbs. The final two fertiliser rates were low N inputs of 180 kg N/ha/year for grasses and herbs and 156 kg N/ha/year for legumes or high N inputs of 450 kg N/ha/year for grasses and herbs and 389 kg N/ha/year for legumes.

Table 4.1 Forage species sown at Lincoln University Research Dairy Farm and their functional groups, scientific name, cultivar and sowing rate.

Forage	Functional group	Scientific name	Cultivar	Sowing rate (kg/ha)
Perennial ryegrass	Grass	*Lolium perenne*	One50 (AR37)	20
Italian ryegrass	Grass	*Lolium multiflorum*	Tabu	25
Chicory	Herb	*Cichorium intybus*	Choice	8
Plantain	Herb	*Plantago lanceolata*	Tonic	10
White clover	Legume	*Trifolium repens*	Kopu II	5
Red clover	Legume	*Trifolium pratense*	Sensation	10

Plate 4.1 Experimental plots at Lincoln University Research Dairy Farm on 29 October 2014

4.2.2 Management

Establishment

The site was sprayed with glyphosate, cultivated with a Duncan Contoura with rear crumbler, power-harrowed using a LELY Power Harrow (2.5 m width) and rolled with a Cambridge Roller in March 2014. All species were sown as monocultures with a Flexiseeder 14-row plot drill (width 2.1 m) on 20 March 2014. Cultivars and sowing rates of each species can be found in Table 4.1. All species were grown under the same climatic and edaphic conditions with soils and nutrient (excluding nitrogen) being non-limiting to growth. The site was irrigated with a travelling irrigator between October and March, with ~20–30 mm water applied per week (total 550 mm).

Feriliser inputs

Soil nutrient sampling was conducted before the experiment began with a soil corer to a depth of 75 mm; results showed pH = 6.1 (soil : water ratio, 1 : 2), Olsen phosphorus (P) = 26 mg/L (Olsen et al. 1954), sulphate-sulphur (sulphate-S) = 5 mg/kg (Watkinson and Kear 1994) and potassium (K) = 0.23 milliequivalents/100 g (Rayment and Higginson 1992). On the basis of this, plots were fertilised with 12.8 kg P/ha, 20 kg K/ha and 32.8 kg S/ha in March and October 2014. Lime was applied in October 2014 at a rate of 2 T/ha.

Weed control

Herbicide T Max (active ingredient aminopyralid) was applied at a rate of 40 mL/10 L water to grass and plantain plots on 22 January and 4 May 2015 to remove dicotyledon species, particularly white clover. Herbicide Gallant (active ingredient haloxyfop-P-methyl) was applied at 5 mL/10 L water with surfactant Uptake (50 ml/20 L water) to legume and herb plots on 4 December 2014, 22 January, 4 May and 10 June 2015 to remove grass species from legume and herb plots.

Herbage removal

Plots were managed by mowing with a Walker MC GHS ride on rotary lawnmower, with mower height set to 4 cm for all species under a cut and carry regime. The timing of harvests was determined by best practices for maximising herbage growth and persistence of the three different functional groups: grasses, legumes and herbs (Lee et al. 2011; Moot et al. 2003). Grasses and herbs were defoliated at 32, 26 and 30 day intervals in spring, summer and autumn, respectively. Legumes were harvested at 41, 35 and 41 day intervals in spring, summer and autumn, respectively. Due to low soil temperatures and slow growth rates, no plots were harvested in winter.

4.2.3 Meterological data

Rainfall and air temperatures for the experimental period are presented in Figure 4.1. Total annual rainfall (386 mm) was lower than the average long term rainfall of the last 30 years (599 mm). This shortfall in rain was compensated with irrigation in the key growing season, which was applied at a rate of 23 mm/week, supplemental to rainfall, between November and March. The monthly air temperatures (Figure 4.1b) showed a similar trend to the long-term average air temperature.

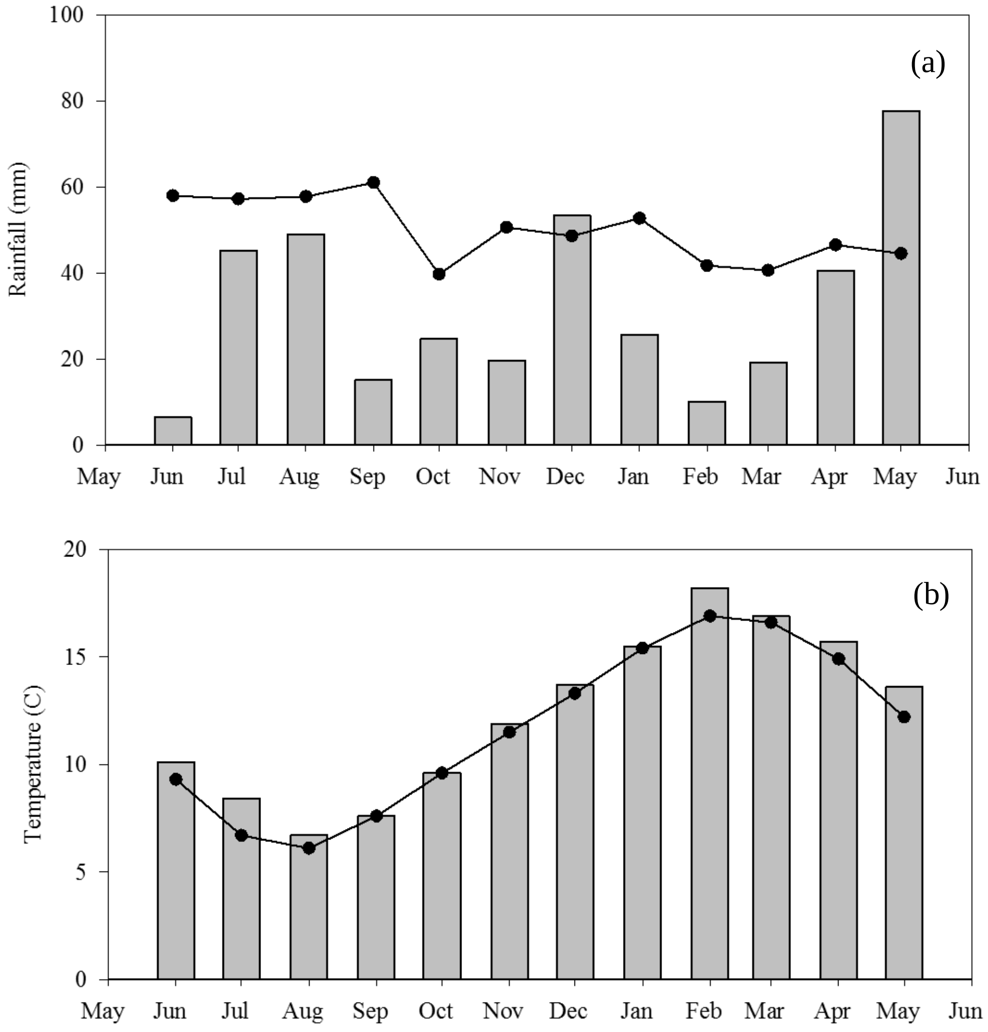


Figure 4.1 (a) Monthly mean monthly rainfall and (b) mean air temperature for June 2014–May 2015 at Lincoln, Canterbury, New Zealand. Data were collected from Broadfields Meteorological Station, 1 km from research site. June 2014–May 2015 (▨), long-term average (1981–2010) (—).

4.2.4 Herbage measurements

At each harvest samples were taken from each plot at 0700 h and 1600 h. A 1 m strip was cut at grazing height (4 cm) using electric hand shears. Each sample was frozen and freeze dried before being ground through a 1 mm sieve using a M200 rotor mill (Retsch Inc. Newtown, Pennsylvania, USA). Ground samples were scanned by near infra-red reflectance spectrophotometer (NIRS, NIRSystems 5000, Foss, Maryland, USA) to determine crude protein (CP), water soluble carbohydrates (WSC), fibre (NDF) and digestibility (DOMD). NIRS calibrations were created using samples from this experiment and from Martin et al. (2017). However, when samples were outside the calibration spectrum, wet chemistry was conducted. Data for each plot were averaged seasonally. Harvests in spring occurred between 9 October 2014 and 30 October 2014. Summer harvests occurred between 24 November 2014 and 10 February 2015 and autumn harvests between 10 March 2014 and 18 May 2015. Dates of each harvest contributing to season data are presented in Appendix B, Table A.5.

4.2.5 Statistical analysis

Data were averaged for each treatment seasonally. Data were analysed by spilt-split plot ANOVA using Genstat (VSN International LTD. 2102) with forage type as the whole plot, N rate as the split plot and time of day as the split-split plot. Differences were considered significant at P<0.05.

A contrast was embedded in the ANOVA treatment structure to compare differences in chemical constituents between functional groups (grasses, herbs and legumes) and within functional groups (perennial vs Italian ryegrass, plantain vs chicory and white vs red clover). This was also carried out to compare differences in the N rate effect and time of day effect between functional groups and forages within functional groups. Contrast results are presented in the Appendix of this thesis.

4.3 Results

Table 4.2 Mean herbage crude protein (CP) concentration (g/kg DM) of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn) in the morning (AM) or afternoon (PM) at low and high N fertiliser inputs

N inputs	Harvest Time	Functional group	Forage	Spring	Summer	Autumn
Low	AM	Grass	Ital Ryegrass	146	137	155
			Per. Ryegrass	158	124	182
		Herb	Chicory	167	180	217
			Plantain	157	142	198
		Legume	Red Clover	244	234	278
			White Clover	257	260	294
	PM	Grass	Ital Ryegrass	121	127	180
			Per. Ryegrass	143	115	166
		Herb	Chicory	166	162	193
			Plantain	145	131	179
		Legume	Red Clover	232	222	259
			White Clover	246	246	277
High	AM	Grass	Ital Ryegrass	198	167	184
			Per. Ryegrass	210	158	220
		Herb	Chicory	188	223	246
			Plantain	186	163	204
		Legume	Red Clover	256	237	272
			White Clover	274	250	295
	PM	Grass	Ital Ryegrass	173	156	207
			Per. Ryegrass	175	155	205
		Herb	Chicory	189	195	221
			Plantain	164	152	191
		Legume	Red Clover	241	228	263
			White Clover	239	245	288
		SEM		2.8	2.2	2.4
		Forage		**<0.001**	**<0.001**	**<0.001**
		N rate		**<0.001**	**<0.001**	**<0.001**
		Forage*N rate		**0.026**	**<0.001**	**<0.001**
		Time		**<0.001**	**<0.001**	**<0.001**
		Forage*Time		0.085	0.08	**<0.001**
		N rate*Time		0.107	0.887	0.222
		Forage*time*Nrate		0.614	0.634	0.822

4.3.1 Crude protein

The effect of time of day and N fertiliser inputs on the CP of forages seasonally are presented in Table 4.2. The CP concentration of the legumes (average 257 g/kg DM) was the highest of all species and was unaffected by N fertiliser. There was no difference in the effects of N fertiliser of time of day between forages within their functional groups. Increasing N fertiliser inputs increased ($P<0.001$) the CP concentration of grasses and herbs by an average of 20 g/kg DM across all seasons. The increase in CP with increasing fertiliser inputs grasses and herbs was lower in spring (+ 13 g CP/kg DM) than summer (+ 30 g CP/kg DM) or autumn (+ 26 g CP/kg DM). Herbs had greater ($P<0.001$) CP concentration (average 184 g/kg DM) than grasses (average 161 g/kg DM) in autumn and summer. Across all seasons the CP concentration of grasses and herbs was reduced on average by 16 g CP/kg DM by harvesting in the afternoon regardless of N fertiliser rate. Chicory tended to have a greater CP concentration than plantain and a greater concentration of CP at the higher N rate in summer. Of the grasses perennial ryegrass had a greater ($P=0.050$) CP concentration than Italian ryegrass, which was consistent across seasons.

4.3.2 Water soluble carbohydrates

Herbage WSC concentrations as effected by harvest time and N fertiliser inputs across seasons is presented in Table 4.3. Increasing N fertiliser inputs reduced ($P=0.030$) the WSC concentration of grasses. The effect was greater for perennial ryegrass than Italian ryegrass. There was no N input effect on WSC concentration for herbs or legumes. White clover, chicory and plantain tended to have a lower WSC concentration than grasses across all seasons and treatments. There was no difference in the WSC concentration between Italian and perennial ryegrass in summer and autumn. The WSC concentration was greater for Italian ryegrass (290 g WSC/kg DM) than perennial ryegrass (243 g WSC/kg DM) in spring.

Across all seasons, there was an increase in the WSC concentration from morning to afternoon for all species which was not affected by N rate. The effect of time of day on WSC concentration was different among forages. Italian ryegrass showed the greatest increase in WSC concentration diurnally (average +90 g WSC/kg DM). On average the concentration of WSC in perennial was 59 g WSC/kg DM greater in afternoon harvested herbage compared with morning harvested herbage. In autumn and spring, the increase in WSC concentration from morning to afternoon was close to twice that as the increase seen for herbs and about three times that seen in white clover. In spring and summer the increase in WSC concentration from morning to afternoon was greater ($P=0.038$) for chicory than plantain.

Table 4.3 Mean herbage water soluble carbohydrate (WSC) concentration (g/kg DM) of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn) in the morning (AM) or afternoon (PM) at low and high N fertiliser inputs

N inputs	Harvest Time	Functional group	Forage	Spring	Summer	Autumn
Low	AM	Grass	Ital Ryegrass	301	273	142
			Per. Ryegrass	230	274	161
		Herb	Chicory	190	152	143
			Plantain	210	179	164
		Legume	Red Clover	177	151	120
			White Clover	194	132	156
	PM	Grass	Ital Ryegrass	365	328	235
			Per. Ryegrass	295	333	234
		Herb	Chicory	241	189	202
			Plantain	227	202	214
		Legume	Red Clover	183	168	146
			White Clover	225	163	193
High	AM	Grass	Ital Ryegrass	217	241	134
			Per. Ryegrass	192	222	142
		Herb	Chicory	186	151	151
			Plantain	202	176	177
		Legume	Red Clover	177	157	131
			White Clover	175	149	165
	PM	Grass	Ital Ryegrass	279	294	223
			Per. Ryegrass	255	259	189
		Herb	Chicory	228	204	204
			Plantain	227	208	203
		Legume	Red Clover	191	173	138
			White Clover	225	166	181
		SEM		4.2	3.2	2.6
		Forage		**<0.001**	**<0.001**	**<0.001**
		N rate		**0.001**	**0.003**	**0.034**
		Forage*N rate		**0.013**	**<0.001**	**0.014**
		Time		**<0.001**	**<0.001**	**<0.001**
		Forage*Time		**0.002**	**<0.001**	**<0.001**
		N rate*Time		0.801	0.568	**0.002**
		Forage*time*Nrate		0.893	0.148	0.711

Table 4.4 Mean herbage neutral detergent fibre (NDF) concentration (g/kg DM) of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn) in the morning (AM) or afternoon (PM) at low and high N fertiliser inputs

N inputs	Harvest Time	Functional group	Forage	Spring	Summer	Autumn
Low	AM	Grass	Ital Ryegrass	389	471	364
			Per. Ryegrass	424	466	455
		Herb	Chicory	183	231	186
			Plantain	248	302	237
		Legume	Red Clover	242	288	252
			White Clover	222	270	231
	PM	Grass	Ital Ryegrass	373	439	401
			Per. s	391	434	431
		Herb	Chicory	158	227	185
			Plantain	221	303	233
		Legume	Red Clover	228	274	251
			White Clover	215	254	218
High	AM	Grass	Ital Ryegrass	404	465	345
			Per. Ryegrass	403	467	443
		Herb	Chicory	176	227	202
			Plantain	228	297	236
		Legume	Red Clover	237	286	257
			White Clover	216	263	225
	PM	Grass	Ital Ryegrass	386	433	398
			Per. Ryegrass	401	442	426
		Herb	Chicory	144	225	186
			Plantain	198	291	240
		Legume	Red Clover	244	276	253
			White Clover	212	259	223
		SEM		6.0	4.4	5.1
		Forage		**<0.001**	**<0.001**	**<0.001**
		N rate		0.074	0.329	0.447
		Forage*N rate		0.007	0.748	0.004
		Time		**<0.001**	**<0.001**	0.667
		Forage*Time		**0.036**	**0.001**	**<0.001**
		N rate*Time		0.134	0.447	0.171
		Forage*time*Nrate		0.102	0.948	0.038

4.3.3 Fibre

The average concentration of NDF was two times greater (P<0.001) for grasses than herbs or legumes across all seasons (Table 4.4). Of the forages, chicory had the lowest (P<0.001) average NDF concentration (average 194 g NDF/kg DM) regardless of treatment or season. Grasses had a similar (P=0.873) NDF concentration in summer averaging 452 g NDF/kg DM. In spring and autumn the NDF concentration was greater (P<0.001) in perennial ryegrass (405 and 439 g NDF/kg DM respectively) than Italian ryegrass (388 and 377 g NDF/kg DM for spring and autumn respectively).

The effect of time of day and N fertiliser on fibre concentration was inconsistent across forage types and seasons. There was no effect on N fertiliser on the NDF concentration for all forages in in autumn and summer. In summer and spring the diurnal reduction in NDF concentration of grasses (average -24 g NDF/kg DM) was the greatest (P<0.001). There was no time of day effect on the NDF concentration of plantain in autumn or spring. The effect of harvest time on NDF concentration in summer was similar (P=0.082) for herbs and legumes.

4.3.4 Digestibility

There was no influence of N fertiliser inputs on DOMD for all species across all seasons (Table 4.5). Across all seasons chicory and white clover had the highest (P=0.002) DOMD regardless of harvest time. There was no diurnal influence on the DOMD of chicory and plantain in spring.

In autumn the diurnal increase in DOMD twice as large for Italian ryegrass (+ 80 g/kg DM), than perennial ryegrass (+29 g/kg DM). There was a larger diurnal increase in DOMD for chicory (+ 24 g/kg DM) than for plantain (+ 14 g/kg DM) in autumn only. There was no difference in the diurnal increase between white and red clover in any season. The diurnal increase in DOMD was similar for chicory and plantain (+ 16 and 10g/kg DM respectively) in summer. This was lower than the diurnal increase in DOMD for grasses (+ 30 g/kg DM) in summer.

Table 4.5 Mean herbage digestibility of organic matter (DOMD) concentration (g/kg DM) of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn) in the morning (AM) or afternoon (PM) at low and high N fertiliser inputs

N inputs	Harvest Time	Functional group	Forage	Spring	Summer	Autumn
Low	AM	Grass	Ital Ryegrass	811	734	639
			Per. Ryegrass	781	753	757
		Herb	Chicory	808	770	812
			Plantain	774	728	775
		Legume	Red Clover	740	701	734
			White Clover	807	753	806
	PM	Grass	Ital Ryegrass	827	767	800
			Per. Ryegrass	808	785	790
		Herb	Chicory	807	783	833
			Plantain	773	733	795
		Legume	Red Clover	762	715	746
			White Clover	822	787	820
High	AM	Grass	Ital Ryegrass	789	735	645
			Per. Ryegrass	787	742	758
		Herb	Chicory	809	775	812
			Plantain	792	740	783
		Legume	Red Clover	762	703	730
			White Clover	816	761	814
	PM	Grass	Ital Ryegrass	800	765	803
			Per. Ryegrass	796	769	783
		Herb	Chicory	817	794	839
			Plantain	788	753	792
		Legume	Red Clover	756	713	748
			White Clover	823	777	823
		SEM		2.0	2.2	3.8
		Forage		**<0.001**	**<0.001**	**<0.001**
		N rate		0.999	0.611	0.190
		Forage*N rate		0.060	0.191	0.506
		Time		**<0.001**	**<0.001**	**<0.001**
		Forage*Time		0.099	**0.024**	**<0.001**
		N rate*Time		0.111	0.587	0.394
		Forage*time*Nrate		0.314	0.708	0.322

Table 4.6 Mean herbage WSC:CP ratio of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn) in the morning (AM) or afternoon (PM) at low and high N fertiliser inputs

N inputs	Harvest Time	Functional group	Forage	Spring	Summer	Autumn
Low	AM	Grass	Ital Ryegrass	2.1	2.0	0.9
			Per. Ryegrass	1.5	2.2	0.9
		Herb	Chicory	1.1	0.8	0.7
			Plantain	1.3	1.3	0.8
		Legume	Red Clover	0.7	0.6	0.4
			White Clover	0.8	0.5	0.5
	PM	Grass	Ital Ryegrass	3.0	2.6	1.3
			Per. Ryegrass	2.1	2.9	1.4
		Herb	Chicory	1.5	1.2	1.0
			Plantain	1.6	1.5	1.2
		Legume	Red Clover	0.8	0.8	0.6
			White Clover	0.9	0.7	0.7
High	AM	Grass	Ital Ryegrass	1.1	1.4	0.7
			Per. Ryegrass	0.9	1.4	0.6
		Herb	Chicory	1.0	0.7	0.6
			Plantain	1.1	1.1	0.9
		Legume	Red Clover	0.7	0.7	0.5
			White Clover	0.6	0.6	0.6
	PM	Grass	Ital Ryegrass	1.6	1.9	1.1
			Per. Ryegrass	1.5	1.7	0.9
		Herb	Chicory	1.2	1.0	0.9
			Plantain	1.4	1.4	1.1
		Legume	Red Clover	0.8	0.8	0.5
			White Clover	0.9	0.7	0.6
		SEM		0.055	0.054	0.022
		Forage		**<0.001**	**<0.001**	**<0.001**
		N rate		**<0.001**	**<0.001**	**<0.001**
		Forage*N rate		**<0.001**	**<0.001**	**<0.001**
		Time		**<0.001**	**<0.001**	**<0.001**
		Forage*Time		**0.009**	0.343	**<0.001**
		N rate*Time		0.429	0.388	**0.019**
		Forage*time*Nrate		0.642	0.888	0.889

4.3.5 WSC:CP

The ratio of WSC to CP for all forages had a large seasonal variation (Table 4.6). Legumes consistently had the lowest ($P<0.001$) WSC:CP which was typically less than half that of grasses. Across all seasons there was no difference ($P=0.796$) in WSC:CP between forages within their functional groups.

Grasses had the highest WSC:CP in spring and summer. Autumn was the only season where the WSC:CP fell below 0.9 for grass samples harvested in the morning in the high N input treatment. For all seasons and forages the WSC:CP ratio was increased ($P<0.001$) by harvesting in the afternoon. At high N inputs the WSC:CP of herbs and grasses was lower ($P<0.001$) than at low N inputs. There was no effect ($P=0.578$) of N inputs on WSC:CP of legumes.

4.4 Discussion

The aim of this study was to investigate the effects of time of day and N fertiliser inputs on chemical composition of four different forage species. Nitrogen inputs had relatively little effects on the chemical composition of the forages. Increasing N fertiliser inputs did not improve DOMD and WSC nor reduce NDF concentration of any of the forages. Unsurprisingly and similar to previous research (Elgersma *et al.* 1997; Martin *et al.* 2017) there was an increase in CP for grasses and herbs when N fertiliser inputs were increased and no effect of N fertiliser on the CP of legumes. This suggests altering N fertiliser inputs is not a beneficial management practice to improve forage quality (DOMD and WSC). The effect of harvest time on WSC, NDF and DOMD of all forages was greater than the effect of N fertiliser.

4.4.1 WSC and CP

Diurnal changes in chemical composition of ryegrass have been previously used to create an increase in the concentration of WSC at the expense of CP with a goal of increasing the utilisation efficiency of dietary N (Miller *et al.* 2001; Moorby *et al.* 2006). The reduction in both CP and NDF as WSC increases would be favourable to animals grazing pastures in New Zealand in most circumstances. Experiments which utilised diurnal changes in plant chemical composition have inconsistently shown reductions in urinary N concentrations (Miller *et al.* 2001; Bryant *et al.* 2014). In this experiment forage types showed differences in their concentration of WSC and their diurnal accumulation of WSC. Italian ryegrass was included in this experiment as it tends to have a higher WSC concentration than perennial ryegrass (Sun *et al.* 2010) and can reduce N leaching losses due to increased N uptake (Moir *et al.* 2013; Malcolm *et al.* 2014; Woods *et al.* 2016). In the current experiment WSC concentration of Italian ryegrass was greater than perennial ryegrass in spring only. While harvesting in the afternoon increased the WSC concentrations of all species, Italian ryegrass had the greatest accumulation of sugars diurnally. The diurnal increase in WSC concentration of grasses did not always translate to larger reductions in CP compared with the other forages. For grasses and herbs the average diurnal change in CP was relatively small (<16 g CP/kg DM), however when combined with the diurnal increase in WSC the WSC:CP ratio was altered.

Edwards *et al.* (2007) suggested a WSC:CP ratio above *c.* 0.7 can lead to a direct reduction in the proportion of N intake excreted in urine. This occurred for grasses and herbs in spring and summer however, the WSC:CP ratio of legumes was typically below 0.7 across all seasons. There was a strong effect of harvesting in the afternoon on the WSC:CP ratio in autumn which was consistent across all species although greatest for grasses. This higher WSC:CP ratio in the afternoon may increase microbial protein synthesis efficiency and nitrogen-use efficiency of dairy cows (Vibart *et al.* 2012), as herbage provides more rapidly fermentable carbohydrates (Hristov *et al.* 2005) and produces less ammonia in the rumen (Tamminga 1996). The larger diurnal accumulation of WSC for grasses suggests that afternoon allocation is more important for ryegrass than chicory or plantain.

4.4.2 Crude protein

Under irrigation, grasses grown in Canterbury may not meet the CP requirements of high producing ruminants in summer. In summer perennial ryegrass at the lower N rate had an average CP concentration of 119 g CP/kg DM which is below the CP requirement for a high producing dairy cow (CSIRO 2007). At this time, increasing N inputs from 180 kg N/ha/year to 450 kg N/ha/year increased the CP concentration of grasses and herbs by an average of 3.2 g CP/kg DM. Chicory and plantain at low N inputs in summer had a higher average CP concentration (153 g CP/kg DM) than perennial ryegrass (119 g CP/kg DM), which is consistent with the results of previous studies (Sanderson *et al.* 2003; Belesky *et al.* 2004). Where N fertiliser use is limited herbs may provide a suitable alternative to meet CP requirements. Chicory had the highest CP concentration regardless of N fertiliser rate or harvest time. This is consistent with findings by Martin *et al.* (2017) who showed chicory had a greater N concentration than plantain or ryegrass across fertiliser inputs ranging from 0 to 450 kg N/ha/year. This may lead to higher N intakes from chicory per unit of DM consumed compared with ryegrass or plantain. Despite this increased CP concentration, grazing experiments which include chicory in the diet of dairy cows have consistently shown a reduction in urinary N concentration (Totty *et al.* 2013; Bryant *et al.* 2017; Minneé *et al.* 2017) which suggests factors other than CP intake may drive lower N excretion in livestock grazing pasture containing chicory.

4.4.3 Fibre

Grasses consistently had the highest NDF concentration, legumes intermediate and herbs lowest. Between the grasses, Italian ryegrass had a lower NDF concentration in spring and autumn which is consistent with previous research (Cosgrove *et al.* 2007b; Sun *et al.* 2010). For chicory, plantain and white clover the proportion of NDF was always below the recommended 35% for adequate rumen function (CSIRO 2007). The lower NDF of chicory and plantain has been seen before in Chapter 3 and previous grazing dairy experiments (Totty *et al.* 2013; Edwards *et al.* 2015; Bryant *et al.* 2017) where milk production was similar or greater, indicating that the low NDF of herbs or mixed swards was not negatively affecting milk production.

Fibre content for all pasture species increased over the summer. This was particularly prevalent in the grass species. For all forage types the proportion of NDF could be reduced by harvesting in the afternoon but could not be altered by N fertiliser. The diurnal reduction in NDF (average 13 g/kg DM) was typically larger than the average reduction in CP (10 g/kg DM). This diurnal reduction in NDF was generally greatest for ryegrass. The reduction in NDF, combined with a reduction in CP offset the increase in WSC diurnally. This contrasts with (Cosgrove *et al.* 2009), who found a diurnal increases in WSC in three ryegrass cultivars was largely offset by reduction in CP and to a lesser extent NDF. Despite the differences both experiments show the potential of increased WSC concentration to enhance the nutritional value of forages, in particular ryegrass.

4.5 Conclusions

- Typically the difference in the nutritive parameters among forages was greater than any diurnal or N fertiliser effect.
- The diurnal effect on chemical composition was greater than the effects of N fertiliser inputs.
- Ryegrass showed the greatest accumulation of WSC concentration diurnally. However, this did not always translate to the largest reduction in CP from morning to afternoon as NDF reduction were typically greater than reductions in CP.
- In autumn there was a strong effect of harvesting forages in the afternoon on WSC:CP particularly for ryegrass. Afternoon allocation of ryegrass may offer a strategy to enhance the nutritive value of forages. Allocation timing appears to be less important for chicory and plantain.

Chapter 5

In sacco digestion kinetics of plantain and ryegrass-white clover harvested in the morning and afternoon

5.1 Introduction

The predominant feed source of dairy cows in New Zealand is perennial ryegrass-white clover sown as a binary mix (Charlton *et al.* 1999). The pasture is productive under grazing, persist well and easy to manage (Kemp *et al.* 1999). However issues arise in terms of nitrogen (N) losses from animals grazing perennial ryegrass-white clover. The pasture supplies grazing cows with N in excess of an animals requirements which is excreted predominantly in the urine (Tamminga 1992; Cameron *et al.* 2013). Alternative forage types to perennial ryegrass-white clover pastures such as plantain are increasing in popularity due to their potential to reduce urinary N losses while maintaining production (Totty *et al.* 2013; Edwards *et al.* 2015; Pembleton *et al.* 2016).

Plantain is a pasture herb which has shown greater dry matter (DM) production that traditional perennial ryegrass-white clover pastures in an irrigated cut and carry experiment (Martin *et al.* 2017). Milk production has also reported to be greater for cows grazing plantain. Chapter 3 found cows grazing pure plantain swards produced 11% more milk than those on perennial ryegrass-white clover in late lactation. Pembleton *et al.* (2016) reported improved milk yield from cattle grazing a mixed sward of perennial ryegrass-clover and plantain compared with those grazing perennial ryegrass-white clover alone in early lactation. Both experiments attributed the increase in milk production to an increase in DM intake despite similar herbage allocations.

One of the mechanisms that controls feed intake is rumen fill (Allen 2014). Forages with lower DM such as plantain (Minneé *et al.* 2013) are typically digested more rapidly than perennial ryegrass (Burke *et al.* 2000). Further, forages with greater fibre content are more slowly digested (John *et al.* 1987; Chaves *et al.* 2006). Fibre concentration, DM and energy of forages also may show diurnal fluctuations. Perennial ryegrass, white clover and plantain harvested in the afternoon had a lower fibre content than morning harvested forage (Cosgrove *et al.* 2009). Chapter 4 showed the fibre content of perennial ryegrass was reduced by ~5% by harvesting in the afternoon as opposed to the morning and plantain fibre which was unaffected time of day. Mechanisms which lower the fibre content of forages may be useful to increase the rate of degradation and in turn improve DM intake. Further, harvest time can have implications on the proportion of crude protein (CP) in the feed. A diurnal increase in water soluble carbohydrates (WSC) often comes at the expense of CP and fibre (NDF) (Cosgrove *et al.* 2007b) which can be beneficial to reduce N losses to the urine and improve milk production through increased DM intake (Miller *et al.* 2001). However the effects of utilising afternoon harvesting of forages on rumen degradation rates is unknown.

The rate and extent of degradation of DM, organic matter (OM) and nitrogen (N) in the rumen has implications on animal production and nitrogen use efficiency (Allen 2014). Adequate balance of OM relative to N available in the rumen has the potential to improve microbial protein synthesis and increase metabolisable protein supply to the ruminant (Tas *et al.* 2006a). Therefore alternative forages, such as plantain, or diurnal allocation strategies which improve the proportion of OM degraded relative to N may be beneficial in improving nitrogen use efficiency in dairy cows. The objective of this chapter was to determine the DM, OM, and N digestion kinetics of plantain cut in the morning and afternoon and compare them perennial ryegrass and white clover.

5.2 Materials and methods

5.2.1 Experimental site and design

Rumen degradation characteristics were determined with nylon bag incubations (Ørskov *et al.* 1980) of plantain and perennial ryegrass-white clover. The experiment was a 2 × 2 factorial design. There were two pasture treatments of either plantain or perennial ryegrass-white clover which were harvested at two times of the day; 0700 hrs or 1600 hours. This created four treatments. Four rumen cannulated multiparous Jersey × Friesian lactating dairy cows were used. Each cow received one treatment, repeated four times until all cows received each treatment. The experiment was carried out at the Lincoln University Research Dairy Farm with the approval of the Lincoln University Animal Ethics Committee (AEC 398) in March 2015.

Throughout the duration of the experiment and for four days prior to the experiment cows were allocated to spatially separate perennial ryegrass-white clover and plantain to achieve a diet of approximately 50% plantain and 50% perennial ryegrass-white clover. This allowed cows to be consuming the same forages that were used for treatments in the nylon bags. Cows were offered a target daily allowance of 30 kg DM/cow/day above ground level, based on energy requirements for milk production. Pre grazing herbage mass was determined using a rising plate meter as describe in Chapter 3 (Section 3.2.2).

5.2.2 Forage preparation

Herbage for rumen incubations was collected from existing paddocks sown with either plantain, cultivar Tonic, or perennial ryegrass, cultivar Arrow with AR1, and white clover, cultivar Kopu II. The perennial ryegrass-white clover pastures were established in March 2014 and the plantain pastures in December 2014. For each treatment four bags of herbage of approximately 1 kg each were harvested to grazing height using hand shears. Harvested herbage was cut to 2 cm lengths to replicate mastication. Herbage of approximately 60 g fresh weight was weighed into individual dacron bags (10 x 15 cm with 50µ pore size). Bags were attached to a galvanised chain and frozen at -16^{o}C until required. Duplicate – and triplicate for the 48 h interval - bags were attached to 1 m length galvanised chain using plastic cable ties. Two subsamples of herbage for each treatment were taken, the first was oven dried at 65^{o}C for 48 h to determine DM % and the second subsample was freeze dried and ground through a 1 mm sieve. Ground material was NIRS scanned to determine chemical composition of the original herbage.

Plate 5.1 Cut herbage in dacron bags attached to alternate galvanised chain links by cable ties.

5.2.3 Rumen incubations

Degradation measurements commenced following morning milking at 0630 hr between 11 and 23 April 2016 using incubation intervals of 3, 6, 9, 12, 24, and 48 h, including wash samples at 0 h. Before incubations, frozen nylon bags with samples were warmed up in water (40°C) for 20 min as suggested by (Sun *et al.* 2012). Following each incubation, bags were plunged into ice water and rumen debris removed followed by washing by hand until water ran clear then drying at 65°C for 48 hours. Total DM was recorded for each bag. Dried herbage material was ground through a 1 mm sieve and analysed for OM (ashed at 500 °C) and N (Elementar Vario MAX) concentration.

5.2.4 Digestion kinetics

Rumen factorial disappearance rate of DM, N, and OM in the rumen was calculated using a nonlinear model (Equation 1) using Sigmaplot 13.0. Effective degradability of DM, N, and OM was calculated using Equation 2 fitted to constant rumen passage rate of 0.06 %/h.

Equation 1 Factorial disappearance, where P= disappearance, a= soluble fraction, b= potentially degradable fraction, e= exponential, c= fractional degradation rate (%/hour), and t= time (hour) (Ørskov *et al.* 2009)

$$P = a \times b \times (1 - e^{-ct})$$

Equation 2 Effective degradation (ED), where P= disappearance, a= soluble fraction, b= potentially degradable fraction, c= fractional degradation rate (%/hour), and k= rumen passage rate (%/hour) (Valderrama *et al.* 2011)

$$ED = a + \frac{b \times c}{c + k}$$

5.2.5 Statistical analysis

A two-way ANOVA (GenStat 15.1) was used to analyse the parameter estimates to determine effects of forage type, harvest time and there interaction. Forage type and harvest time were fixed terms and cows were replicates for the degradation parameters estimated from the non-linear fits.

5.3 Results

The chemical composition of perennial ryegrass-white clover and plantain harvested in the morning and afternoon are shown in Table 5.1. The proportion of fibre (NDF) was 36% lower ($P<0.001$) in plantain than perennial ryegrass-white clover. The effect of time of day on the fibre content of herbage was greater for perennial ryegrass-white clover than plantain. The NDF concentration of pasture was reduced by 8% by afternoon harvesting. The diurnal increase in DOMD was two times as large ($P=0.047$) for perennial ryegrass-white clover than plantain. While the difference in DOMD between plantain and perennial ryegrass-white clover harvested in the morning was significant ($P=0.013$), the difference was small (0.5%). The DM% of plantain was two thirds that of perennial ryegrass-white clover and had a lower diurnal increase.

Table 5.1 Forage dry matter (DM) content, chemical composition (% of DM) and predicted metabolisable energy (ME) determine by Near Infrared Reflectance Spectroscopy of fresh plantain and perennial ryegrass-white clover harvested in the morning or afternoon

	Plantain		Ryegrass-white clover				
	AM	PM	AM	PM	P pasture	P time	P P*T
DM	8.46	11.6	12.7	19.1	<0.001	<0.001	<0.001
ADF	21.3	21.3	24.2	21.2	<0.001	<0.001	<0.001
WSC	14.9	20.2	8.36	13.2	<0.001	<0.001	0.623
DOMD	73.4	76.4	73.9	79.6	0.013	<0.001	0.047
ME	11.7	12.2	11.8	12.7	0.013	<0.001	0.047
NDF	26.5	25.7	42.6	39.2	<0.001	<0.001	0.012
OM	86.1	87.2	91.2	91.0	<0.0013	0.032	0.003
CP	27.5	24.6	25.3	27.1	0.596	0.058	<0.001

5.3.1 DM digestion kinetics

The *in sacco* DM disappearance data are illustrated in Figure 5.1a. From hours 6 to 24 after incubation DM disappearance was greater ($P<0.001$) for plantain than perennial ryegrass-white clover. DM disappearance tended to be greater for pasture harvested in the afternoon than those in the morning until 24 hours after incubation. Estimated DM fractions and degradation parameters are summarised in Table 5.2. The percentage of DM released during hand washing of un-incubated samples (soluble DM; fraction A) was greater for samples cut in the afternoon regardless of forage type. The fractional DM degradation rate per hour was greater for plantain compared with perennial ryegrass-white clover. There was no effect of harvest time on the fractional degradation rate per hour of DM.

Table 5.2 Mean dry matter (DM), organic matter (OM) and nitrogen (N) degradation characteristics of perennial ryegrass-white clover and plantain harvested at 0700 (AM) or 1600 (PM) defined as soluble fraction (a), potentially soluble fraction (b), fractional disappearance rate (c), potentially degradable (PD), effective degradability at 0.06 h (ED) and effective rumen degradable protein (ERDP).

	Plantain		Ryegrass-white clover				
	AM	PM	AM	PM	P pasture	P time	P p*t
DM							
a	16.4	23.7	14.4	26.6	0.886	**0.010**	0.425
b	74.8	68.7	77.0	64.5	0.795	**0.032**	0.409
C (%/hr)	12.5	11.9	8.01	9.58	**0.050**	0.761	0.487
PD (%)	91.3	92.4	91.4	91.2	0.827	0.859	0.785
ED (%)	66.7	68.7	57.6	65.1	**<0.001**	**0.003**	**0.047**
OM							
a	20.5	24.5	17.4	26.8	0.891	0.090	0.452
b	68.5	65.8	74.0	63.0	0.664	0.121	0.318
c	10.3	10.1	7.66	9.36	0.439	0.726	0.662
PD (%)	88.9	90.3	91.4	89.9	0.576	0.968	0.529
ED (%)	66.5	68.0	58.2	64.2	**<0.001**	**0.005**	**0.043**
N							
a	2.4	10.3	14.6	20.2	**0.005**	**0.049**	0.704
b	93.9	85.5	80.9	75.3	**0.042**	0.188	0.784
c	8.84	8.56	7.09	8.27	0.324	0.656	0.470
PD (%)	96.3	95.8	95.5	95.5	0.846	0.931	0.938
ED (%)	58.0	60.0	57.8	61.8	0.608	0.086	**0.530**
ERDP	151	136	151	162	**0.004**	0.575	**0.006**

5.3.2 OM digestion kinetics

There was little effect of pasture type or time of day on the *in sacco* disappearance of OM as shown in Figure 5.1b. The soluble fraction of OM was greater for samples harvested in the afternoon and was not different between forage types (Table 5.2). There was no effect of forage type or time of harvest on fractions a, b or c nor was there any effect on the potential degradability of OM. The effective degradability was greater for plantain (average 67.3%) than perennial ryegrass-white clover (61.2%). Perennial ryegrass-white clover harvested in the afternoon had a greater effective degradability of 64.2% than perennial ryegrass-white clover harvested in the morning (58.2%). There was no time of harvest effect on plantain samples.

5.3.3 N digestion kinetics

From hours 6 to 24 after incubation, N loss was greater ($P<0.001$) for plantain than perennial ryegrass-white clover and greater for pastures harvested in the afternoon than those harvested in the morning (Figure 5.1c). The soluble and potentially soluble fraction of N was greater ($P<0.05$) for plantain than perennial ryegrass-white clover (Table 5.2). Forages harvested in the afternoon had a greater soluble fraction of N than those harvested in the morning. There was no effect of forage type or time of day on the fractional degradation rate, potential degradability or effective degradability of N.

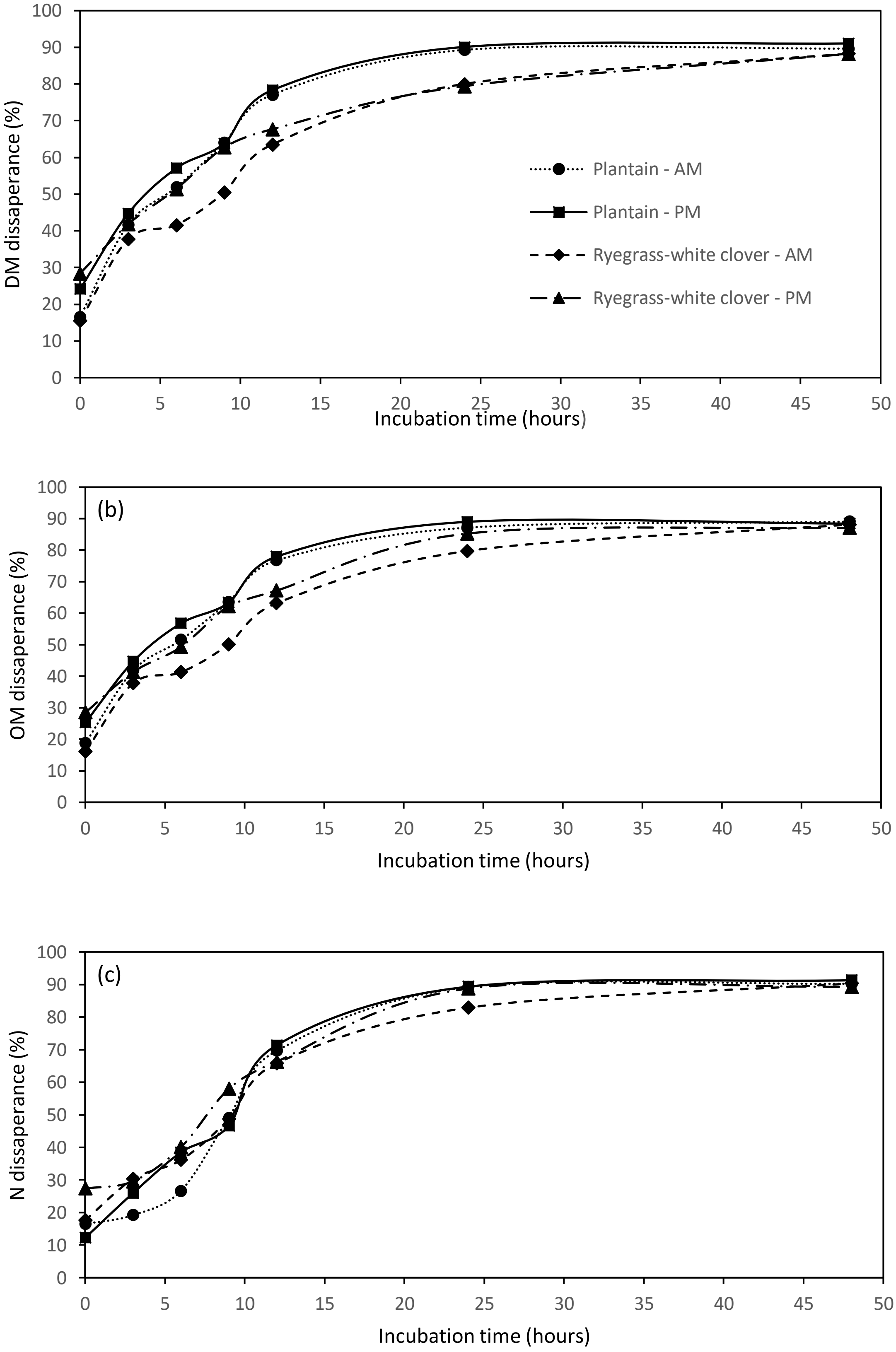


Figure 5.1: *In sacco* disappearance of dry matter (DM) (a), organic matter (OM) (b) and nitrogen (N) (c) for perennial ryegrass-white clover and plantain harvested at 0700 (AM) or 1600 (PM).

5.4 Discussion

5.4.1 Forage effect

This experiment aimed to compare the *in sacco* digestion kinetics of plantain and perennial ryegrass-white clover harvested in the morning or afternoon. Chapter 3 found cows on plantain had increased DM intakes compared with those on perennial ryegrass-white clover in late lactation, despite similar allocations which resulted in improved milksolids production. Similar results have been found by Pembleton *et al.* (2016) who used plantain as an alternative grazed pasture and attributed greater animal production compared with perennial ryegrass-white clover to increased DMI, as allocations were similar. In the current experiment from hours 6 to 24, more plantain DM had disappeared than perennial ryegrass-white clover. Further the rate of DM degradation of plantain was faster than that of perennial ryegrass-white clover. Although degradation rates in this experiment were much slower the than Burke *et al.* (2000) found, the trend for plantain to have a faster rumen clearance time (25%/hr) compared with perennial ryegrass (11%/hr) was similar. The authors attributed the increased rumen clearance time of plantain to the structure of the plant cells and their rumen degradation characteristics. Plantain is known to contain significant concentrations of pectin, a structural but readily fermentable carbohydrate (Judson *et al.* 2009). This result confirms that plantain may be useful to increase DM intake by improving rumen clearance times.

Cows grazing pastures which include plantain have reduced urinary N concentrations (Box *et al.* 2016; Bryant *et al.* 2017; Minneé *et al.* 2017). Minneé *et al.* (2017) suggested the lower CP released from plantain in the early stages of *in sacco* degradation compared with perennial ryegrass may have contributed to lower urinary N. In the current experiment the soluble fraction (A) of N was greater in perennial ryegrass compared with plantain which was similar to the results found by (Minneé *et al.* 2017). However, there was no difference in the rate of degradation of N between perennial ryegrass-white clover and plantain. Further, from 6 to 24 hours after incubation the proportion of N which had disappeared from plantain was greater than that from perennial ryegrass-white clover which was not expected. Unlike Minneé *et al.* (2017) the N content of plantain and perennial ryegrass-white clover was similar. Therefore the greater N disappearance of plantain compared to perennial ryegrass-white clover was due to the increased rate of DM disappearance. For example, from hours 6, 9, 12 and 24, more DM had disappeared for plantain than perennial ryegrass-white clover which coincided with the greater N loss. Typically increased rumen degradation of N leads to greater losses of N via NH_3 and the urine. However plantain appears to not fit this trend. While this experiment showed increased N degradation rates for plantain, a consistent reduction in the concentration of N in the urine has been found for cows grazing pastures containing plantain (Totty *et al.* 2013; Edwards *et al.* 2015; Pembleton *et al.* 2016; Bryant *et al.* 2017). This may be due to changes in the supply of energy in the rumen to better make use of the N from plantain. Navarrete *et al.* (2016) found that the presence of acteoside decreased net NH_3 production due to an increase in fermentable carbohydrates. Ammonia utilisation by microbes in the rumen is primarily carbohydrate-limited (Russell *et al.* 1992). Navarrete *et al.* (2016) concluded that microbes in the rumen were able to utilize acteoside as an energy source thereby incorporating more NH_3 into microbial protein. This suggests that despite a higher solubility of N from plantain there may not be greater N loss in the urine compared with perennial ryegrass-white clover.

5.4.2 Time of day effects

While the forage type had a greater effect on the effective degradability and rate of degradation of DM and OM, the time of day that forages were harvested appeared to have a greater effect on the A and B digestion fractions of DM. Diurnally there was an increase in the A fraction of DM for perennial ryegrass-white clover and plantain and a reduction in the B fraction. This meant that overall there was no time of day effect on the potentially degradable DM of perennial ryegrass-white clover or plantain. This result was not surprising given there were only small increase in WSC and therefore small reductions in NDF. In this experiment concentrations of WSC were 5.3% DM higher for perennial ryegrass-white clover and 4.9 % DM higher for plantain harvested in the afternoon than when harvested in the morning. The magnitude of the difference was smaller than the means reported by Cosgrove *et al.* (2007a) of 6.1 % DM for autumn harvested perennial ryegrass.

Despite no difference in the potential degradability of OM or DM between forage cut in the morning or afternoon, the effective degradability was improved when perennial ryegrass-white clover and plantain were harvested in the afternoon. This diurnal effect was greater for perennial ryegrass-white clover than plantain. This was likely due to perennial ryegrass-white clover having larger changes in chemical composition diurnally. Notably the diurnal reduction in fibre was greater for perennial ryegrass-white clover than that for plantain. This is similar to results found in Chapter 4 and suggests when including pure swards of plantain in a system, management strategies which allow allocation of perennial ryegrass-white clover in the afternoon and plantain in the morning may provide benefits in milk production, through increased DM intake.

5.5 Conclusions

- Forage type had a greater effect on the degradation rate of the two pasture types than harvest time.
- Plantain had faster DM degradation rates than perennial ryegrass-white clover which resulted in faster N degradation rates. As a result the use of plantain as pasture for dairy systems may be useful to increase total voluntary intake.
- Despite some effect of time of day on the nutritive value of perennial ryegrass-white clover and plantain, there was no time of day effect on the potentially degradable DM of the forages.
- Effective degradability was improved when perennial ryegrass-white clover and plantain were harvested in the afternoon. This diurnal effect was greater for perennial ryegrass-white clover than plantain.
- This experiment suggests there would be more benefit from allocating perennial ryegrass-white clover in the afternoon than plantain.

Chapter 6

Milk production from late lactation cows grazing temporally and spatially separated monocultures of plantain and perennial ryegrass-white clover

6.1 Introduction

Perennial ryegrass-white clover pasture is the major forage source for temperate dairy systems (Holmes *et al.* 2007). Recently, there has been increasing interest in using plantain (*Plantago lanceolata*) as an alternative pasture species due to growing evidence that it contributes to reduced urinary nitrogen (N) loss when used as a grazed forage (Woodward *et al.* 2012; Totty *et al.* 2013; Bryant *et al.* 2017) or silage supplement (Judson *et al.* 2016).

Agronomically, plantain has a similar annual dry matter (DM) yield and seasonal growth distribution compared with perennial ryegrass-white clover pastures (Minneé *et al.* 2013). Like perennial ryegrass-based pastures, plantain can be grazed in-situ, allowing the species to fit within a grazing system (Stewart 1996). Best practices for successful establishment and maximum herbage production suggest plantain should be grown as a monoculture (Stewart 1996; Stewart *et al.* 2014). To maximise benefits from plantain a high proportion in the diet is desirable. Chapter 3 demonstrated that early and late lactation cows grazing pure plantain pastures had increased milk production and lower urinary N concentration compared with cows grazing perennial ryegrass and white clover pastures. A high proportion of plantain (~50%) was required in the diet to achieve a 30% reduction in urinary N concentration. However, the effects of allocation methods which achieved a 50-50 perennial ryegrass-white clover-plantain are unknown.

One method for sustaining a 50% perennial ryegrass-white clover and 50% plantain diet is to offer forages separately in the same paddock allowing animals to select their own diet (Edwards *et al.* 1993; Edwards *et al.* 2008). Under this condition, the proportion of plantain on offer can be more easily controlled than when sown in a mixture. However, pure swards of plantain and perennial ryegrass-white clover in the same paddock may be difficult to manage, alternative herbage allocation methods are sought. Previous attempts to increase the proportion of clover in the diet have utilised a temporal grazing system where pure forages are offered at different times of the day. Cosgrove *et al.* (2006) and Nuthall *et al.* (2000) observed an increase in milk solids yield when white clover was offered following morning milking with pure swards of perennial ryegrass offered following afternoon milking as opposed to a perennial ryegrass-white clover mixture. This novel method of allocation (temporal separation) allowed easy control of the proportion of each species in the animals' diet. The effects of novel methods of allocation of plantain and perennial ryegrass-white clover on milk production and urinary N concentration are currently unknown.

Chapter 4 characterised the effects of time of day on the nutritive value of six forages. The experiment found that there was a greater diurnal effect on perennial ryegrass than plantain. The accumulation of sugars diurnally was greater for perennial ryegrass than plantain which led to an improved water soluble carbohydrate (WSC) to crude protein (CP) ratio when perennial ryegrass is harvested in the afternoon. The greater WSC:CP ratio in perennial ryegrass harvested in the afternoon may be beneficial to improve nitrogen use efficiency (NUE) (Edwards *et al.* 2007). Further, Chapter 5 found that harvesting in the afternoon had larger effect on improving the effective degradability for perennial ryegrass-white clover than plantain. The diurnal improvement in effective degradability of perennial ryegrass-white clover was attributed to a diurnal reduction in fibre. These results suggest when including pure swards of plantain in a system, management strategies which allow for allocation of perennial ryegrass-white clover in the afternoon and plantain in the morning may provide the most benefits in terms of improving milk production while reducing urinary N.

The objectives of this experiment were to examine the effects of temporal and spatial separation of equal proportions of pure plantain and perennial ryegrass-white clover pasture on milksolids production and nitrogen excretion in milk, urine and faeces for late lactation dairy cows.

6.2 Materials and methods

6.2.1 Experimental site

The experiment was conducted at the Lincoln University Research Dairy Farm with the approval of the Lincoln University Animal Ethics Committee (AEC 2016-03). The 4.5 ha experimental area used was the same as described in Chapter 3. Before the experiment the pasture was managed by rotational grazing with dairy cows.

6.2.2 Experimental design

The experiment was a completely randomised design consisting of two pasture treatments with three replicates. Both pasture treatments offered 50% of the diet as perennial ryegrass and 50% as plantain, however in the first treatment the two forages were offered in adjacent monocultures (spatial separation) while in the second treatment plantain was offered first (following am milking) and perennial ryegrass-white clover offered in the afternoon (temporal separation).

Twenty four pregnant, lactating Friesian x Jersey dairy cows were blocked according to milksolids production (1.61 ± 0.3 kg MS/cow/d), age (6.9 ± 0.18 years), days in milk (185 ± 4.0 days) and liveweight (536 ± 9.9 kg) (all mean ± sem). Animals were assigned from within blocks to graze one of two pasture treatments in three (1.5 ha paddock) replicates of 4 cows per herd. Cows grazed pastures for a period of 14 days in autumn from 1 to 14 March 2016 which represented a 7 day transition and 7 day experimental period. Before the experiment, all cows rotationally grazed perennial ryegrass-white clover pasture together. Cows were offered a target daily herbage allowance of 30 kg DM/cow/day above ground level, based on energy requirements for milk production. During the experiment, allocation of herbage was based on plate meter manufacturer settings of herbage mass (kg DM/ha) for perennial ryegrass-white clover (140 × RPM reading +500) and previously derived calibration equations between herbage mass and height for plantain (94 × RPM reading + 455) (Haultain *et al.* 2014). The calculated area to be grazed controlled by temporary electric fencing. Each daily allocation was back-fenced to prevent grazing of residual regrowth.

Cows were milked twice daily at approximately 0600 and 1400 h. Cows on spatial separation were offered 100% of their allocation following morning milking while those on temporal separation were offered 50% of their allocation following morning milking (as plantain) and 50% following afternoon milking (as perennial ryegrass). Cows had *ad lib* access to water through portable troughs.

6.2.3 Herbage measurements

Herbage mass

Pasture compressed height, for determination of herbage mass, was measured daily pre- and post-grazing from 50 readings for each grazed area using a rising plate meter (RPM; Jenquip, Filip's EC 09, Electronic Folding plate meter). Apparent DM intake was calculated using calibration equations derived during the experiment. Calibration measurements were collected from forages every second day during the experiment by cutting two 0.2 m^2 quadrats to ground level before and after grazing for each group. Two RPM measurements were recorded in each quadrat prior to harvesting herbage. Collected herbage samples were weighed fresh and a subsample taken to determine botanical composition of forages. Subsampled herbage was sorted into sown grass, white clover, weeds and dead material for perennial ryegrass-white clover pastures and plantain, weeds and dead material for plantain. All botanical components and bulk samples were oven dried at 65^{o}C for 48 h and dry weight and DM% determined.

Linear and curvilinear relationships between herbage mass and pasture height were compared and best-fit equations (greatest r^2) were fitted to the data. The calibration equations for each pasture type were: perennial ryegrass-white clover (kg DM/ha) = 124 × height + 364, $r^2 = 0.61$, and plantain (kg DM/ha) = 97 × height + 491, $r^2 = 0.72$. Apparent group DM intake of cows was calculated from herbage disappearance between pre- and post-grazing calibrated RPM measurements and areas allocated.

Herbage chemical composition

A further herbage sample was collected for each forage type when cows were offered a new break for each pasture type. These herbage samples were taken at 10 random locations across the break above 4 cm pre- and post-grazing at approximately 0700 h for plantain and perennial ryegrass-white clover in the spatial treatment and plantain in the temporal treatment. Herbage samples from perennial ryegrass-white clover in the temporal treatment were collected at approximately 1600 h. Post-grazing samples were considered as anything refused above grazing height (4cm). The samples were immediately frozen before being freeze dried. The dried sample was ground through a 1 mm sieve and scanned by near infra-red spectrophotometer (NIRS, NIRSystems 5000, Foss, Maryland USA) using previous derived calibrations for perennial ryegrass-white clover and plantain to determine crude protein (CP), digestible organic matter (DOMD), water soluble carbohydrate (WSC), acid detergent fibre and neutral-detergent fibre (NDF). Metabolisable energy (ME) was calculated as MJ ME/kg DM =

0.16 × DOMD (CSIRO, 2007). Apparent nutrient intake of each group of cows was calculated from nutrient disappearance between pre- and post-grazing for each pasture type where pre and post-grazing nutrient availability was the concentration of the nutrient multiplied by the pasture mass pre and post-grazing. Ground samples were then subsampled and analysed for mineral composition by inductively coupled plasma atomic emission spectroscopy and secondary plant compounds (catalpol, aucubin and acteoside) analysed for plantain only using high performance liquid chromatography by Massey University analytical laboratory following procedures of Tamura *et al.* (2002).

6.2.4 Animal measurements

Milk production

Milk yield was measured daily for individual cows with an automated system (DeLaval Alpro Herd management system, DeLaval, Tumba Sweden). Two milk samples were collected every second day from every cow at the afternoon and following morning milking to determine milk composition and milk urea nitrogen (MUN). The sample for milk composition was analysed by Livestock Improvement Corporation Ltd (Christchurch, New Zealand) to determine milk fat, protein and lactose by MilkoScan (Foss Electric, Hillerod, Denmark). The second sample for MUN was centrifuged at 4,000 × *g* for 10 min at room temperature and refrigerated for 10 min to allow the fat to solidify on the top and be removed. Skim milk was pipetted into a clean micro-centrifuge tube, frozen before analysis. Skim milk was analysed by Lincoln University Analytical Services by automated Modular P analyser (Roche/Hitachi).

Urine and faecal composition

Spot samples of urine and faeces were collected on days 4, 6 and 8 from all cows following consecutive morning and afternoon milkings. Urine and faecal samples were stored frozen (-20°C). After thawing, each faecal sample was mixed thoroughly and a subsample of approximately 20 g was weighed fresh, oven-dried at 100°C and then weighed dry to determine DM%. Another subsample (~30 g) was freeze-dried for five days at 0.5 mbar, ground through a 1 mm sieve and analysed for N% by Elementar (Variomax CN Analyser. Analysensysteme GmbH, Hanau, Germany). Urine samples were thawed at 4°C and analysed for N% (Elementar).

Urine volume

To measure urination patterns, a urine meter harness as described by Ravera *et al.* (2015) was attached to a random selection of at least five cows each day for 24 hour periods from days 7 to 13 of the experiment. The harness incorporates a flow meter which is glued to the vulva and is attached by wires to a data logger which is worn by the cow in a cover. Movement of cows resulted in frequent failure of the harness due to wire breakages or detachment of the flow meter from the vulva. Although the harness was attached to 48 cows over seven experimental days, breakages restricted the number of cows which were able to provide useable data. A total of 20 cows contributed data to average urination volume, 14 cows provided a full data set from morning to evening milking, twelve cows provided data from evening to morning milking and nine provided 24 hours of data.

6.2.5 Statistical analysis

Herbage and animal data excluding urination behaviour, were analysed using one-way ANOVA in GenStat 15.1 (VSN International LTD. 2012) with replicates as random effect and pasture treatment as fixed effect. Data were average over days across groups for milk variable before analysis. Urination behaviour data was analysed using one-way ANOVA with cows as random effect and treatment as fixed effect. Differences were considered significant at $P<0.05$.

6.3 Results

6.3.1 Herbage

The chemical and botanical constituents of the plantain and perennial ryegrass-white clover offered temporally or spatially are shown in Table 6.2. There was no treatment effect on pre-grazing herbage mass of plantain, however in the spatial treatment pre-graze herbage mass of perennial ryegrass-white clover was 265 kg DM/ha greater than in the temporal treatment ($P<0.05$). The DM and OM contents were lower in plantain than perennial ryegrass-white clover and there was no effect of separation treatment on DM or OM content. The difference in herbage mass of the perennial ryegrass resulted in interactions between plant type and separation treatment for chemical composition. Crude protein content was lowest in plantain, which was not affected by separation treatment. Temporally separated perennial ryegrass-white clover had a higher ($P<0.001$) crude protein (19.2%) than spatially separated perennial ryegrass-white clover (14.6%). The concentration of WSC was lower ($P<0.001$) in temporally separated perennial ryegrass-white clover and the concentration of CP greater ($P<0.001$) compared with spatial separation. Cows grazed plantain to a lower post-grazing herbage mass than perennial ryegrass-white clover, though post-grazing herbage mass was similar between allocation treatments.

Final daily herbage allocation, for both temporal and spatial separation, was lower than the target 30 kg DM/cow/d at 26.4 ± 0.32 kg DM/cow/d above ground level. Apparent DM intake was similar between treatments at approximately 15.0 kg DM/day (Table 6.2). Cows grazing temporally separated forages consumed a higher ($P=0.010$) proportion of their apparent DM intake as plantain (59%) compared with those grazing spatially separated forages (53%).

Table 6.1 Apparent intake of total dry matter (DM), plantain DM, perennial ryegrass-white clover (pasture) DM, water soluble carbohydrate (WSC) crude protein and metabolisable energy (ME) by cows grazing either temporally or spatially separated perennial ryegrass and plantain. LSD = least significant difference ($\alpha=0.05$).

	Temporal		Spatial		Pt	Pf	Pt*f
	Pasture	Plantain	Pasture	Plantain			
Apparent DM intake (kg)	6.20	8.73	7.11	7.97	0.832	<0.001	0.016
WSC intake (kg)	0.53	1.88	1.28	1.76	<0.001	<0.001	<0.001
Crude Protein intake (kg)	1.63	1.07	1.24	0.96	0.002	<0.001	0.081
ME intake (MJ)	76.4	104	88.7	94.7	0.689	<0.001	0.011

T, allocation type; F, forage type

Table 6.2 Mean herbage characteristics and chemical composition at grazing height of plantain and perennial ryegrass-white clover (pasture) for temporal and spatial separation treatments.

	Temporal		Spatial		P t	P f	P t*f
	Pasture	Plantain	Pasture	Plantain			
Pre grazing							
Height	18.7	26.5	20.8	26.3	0.219	<0.001	0.130
Herbage mass (kg DM/ha)	2 664	3 068	2 929	3 046	0.127	0.001	0.072
Plantain (%)	0	75.4	0	73.0	0.462	<0.001	0.368
Grass (%)	54.9	0	62.0	0	0.043	<0.001	0.043
Legume (%)	17.0	1.41	12.5	1.26	0.088	<0.001	0.109
Plantain stems (%)	0	10.7	0	16.1	0.034	<0.001	0.024
Weed (%)	3.01	0.66	3.53	0.34	0.940	<0.001	0.614
Dead (%)	25.2	11.9	22.0	9.30	0.090	<0.001	0.818
DM%	23.5	16.1	21.9	16.0	0.191	<0.001	0.227
OM (%)	91.1	88.4	91.9	88.4	0.054	<0.001	0.071
NDF (%)	38.8	30.0	43.9	29.8	0.002	<0.001	<0.001
ADF (%)	22.9	22.2	25.4	21.5	0.027	<0.001	<0.001
CP (%)	19.2	12.1	14.6	12.2	<0.001	<0.001	<0.001
WSC (%)	17.3	21.8	21.1	22.0	<0.001	<0.001	0.001
ME (MJ ME/kg DM)	12.2	11.9	11.9	11.4	0.164	0.119	0.764
Post grazing							
Height	8.90	6.95	7.94	7.28	0.232	<0.001	0.016
Herbage mass (kg DM/ha)	1 393	1 168	1 332	1 200	0.706	<0.001	0.234

T, allocation type; F, forage type

The mineral composition of perennial ryegrass-white clover and plantain is shown in Table 6.3. The mineral concentration was greater for plantain than perennial ryegrass-white clover for all measured minerals. Sodium concentration was two times greater ($P<0.001$) in plantain than perennial ryegrass-white clover in late lactation only. Allocation method of the forages had little effect on the mineral concentration of herbage. Of the secondary plant compounds measured in plantain acteoside had the highest concentration (Table 6.3).

Table 6.3 Mean herbage mineral composition (% DM) of plantain and perennial ryegrass-white clover (pasture) and bioactive glycoside (mg/g dry DM) plantain.

	Temporal		Spatial		P t	P f	P t*f
	Pasture	Plantain	Pasture	Plantain			
Sodium	0.542	0.866	0.554	0.822	0.686	<0.001	0.491
Magnesium	0.293	0.170	0.235	0.172	0.004	<0.001	0.004
Calcium	0.868	2.12	0.675	2.04	0.025	<0.001	0.314
Chloride	1.95	2.43	1.46	2.63	0.508	<0.001	0.111
Sulphur	0.340	0.352	0.258	0.370	0.177	0.014	0.040
Potassium	2.92	2.23	2.27	2.23	0.010	0.005	0.010
Phosphorus	0.305	0.368	0.340	0.368	0.230	0.004	0.230
DCAD	220	46.3	243	-42.3	0.641	0.004	0.429
K/Na Ratio	6.17	2.67	4.33	2.67	0.198	0.001	0.198
Catalpol		0.409		0.355	0.281		
Aucubin		9.49		8.88	0.553		
Acteoside		36.9		33.5	0.703		

6.3.2 Animal

Milk yield and milk solids were greater ($P<0.001$) for cows grazing spatially compared with temporally separated forages (Table 6.4). There was no effect of treatment on the concentration of fat and protein in milk. Therefore cows grazing spatially allocated forages produced more ($P<0.001$) milk protein and milk fat than those grazing temporally allocated forages (Table 6.4) by virtue of milk volume alone. The lactose concentration and yield were greater for cows grazed spatially than temporally allocated forages.

Table 6.4 Mean milk yield, composition and nitrogen use efficiency (NUE) of dairy cows grazing temporally or spatially separated perennial ryegrass-white clover and plantain (n=4). LSD = least significant difference (α=0.05).

	Temporal	Spatial	LSD	P
Milk Volume (L)	14.7	16.0	0.67	<0.001
Milksolids (kg)	1.37	1.53	0.062	<0.001
Milk Protein (%)	4.17	4.25	0.118	0.138
Milk Protein (kg)	0.61	0.68	0.028	<0.001
Milk Fat (%)	5.41	5.53	0.233	0.320
Milk Fat (kg)	0.76	0.85	0.040	<0.001
Lactose (%)	4.83	4.91	0.047	<0.001
Milk lactose (kg)	0.71	0.78	0.033	<0.001
MUN (mmol/L)	4.37	4.50	0.419	0.616
Milk N output (g N/cow/d)	97.1	108.3	4.45	<0.001
NUE milk (%)	21.7	29.7	2.87	<0.001

There was no effect of treatment on urine or faecal N concentration (Table 6.5). Cows grazing temporally consumed 98 g day more (P<0.001) N than those grazing spatially allocated forages. Nitrogen use efficiency (NUE) (g N milk/g N intake) was greater (P<0.001) for cows grazing spatially (29.7%) than those grazing temporally allocated forages (21.7%).

The mean volume of each urination event was similar between treatments at 2.92 L and 2.74 L for temporal and spatial separation, respectively (Table 6.5). There was large variation among animals in the volume and frequency of urination events. The minimum volume per urination for each cow ranged from 0.51 to 2.57 L, while the maximum volume per urination ranged from 2.14 to 9.30 L. Of the cows which wore the harness for a 24 h period, the total mean daily urine volume was 43 L/cow/d for temporal and spatial treatments, and there was no difference in urination frequency between treatments (13 events/cow/d). Total urination volumes tended to be greater between overnight (average 24.5) than during the day (16.8 L). All urination events where volumes were greater than 5 L occurred between 2100 and 0430 h.

Table 6.5 Mean urination behaviour and urine and faecal N concentration from dairy cows grazing temporally or spatially separated perennial ryegrass-white clover and plantain (n=4). LSD = least significant difference (α=0.05).

	Temporal	Spatial	LSD	P value
Urine				
Daily average N concentration (g N/L)	2.58	3.00	0.047	0.096
Volume (L)				
Total daily volume[1]	41.4 (4)	44.5 (5)	15.02	0.638
Average volume/urination[2]	2.92 (128)	2.74 (147)	0.859	0.670
Frequency (urination/cow/d)[1]	13.5 (4)	13.4 (5)	2.825	0.936
Faecal N (%)	3.14	3.23	0.081	0.048

[1] Values in parenthesis are the total number of cows on which urine meter data was recorded.

[2] Values in parenthesis represent the total number of measurements from the urine meter.

6.4 Discussion

6.4.1 Milk production

The aim of this study was to investigate effects of herbage allocation of a 50-50 perennial ryegrass-white clover-plantain diet on milk production of grazing dairy cows. This experiment was designed to test the effects of spatial versus temporal separation of perennial ryegrass-white clover with pure plantain on milk production and urinary N. Chapter 3 showed a diet of 50% perennial ryegrass-white clover and 50% pure plantain allocated as spatial separated forages reduced urinary N concentration of dairy cows by over 30% compared with perennial ryegrass-white clover pastures. However, due to differences in residuals and regrowth, pastures sown as spatially separated monocultures may be difficult to manage. In this experiment under equal herbage allocation cows grazing spatially separated perennial ryegrass-plantain produced 12% more milksolids than those grazing temporally separated perennial ryegrass-plantain. This contrasts with previous studies where temporal separation of perennial ryegrass and white clover gave 14% greater milk production (Rutter *et al.* 2003). However, unlike the current experiment, Rutter *et al.* (2003) only compared allocation of perennial ryegrass and white clover (as monocultures) and did not include an alternative forage (plantain).

Typically milk production is driven by ME intake when protein is not limiting (CSIRO 2007). In this experiment, allocation method did not affect apparent DM or ME intake (averaging 15 kg DM/cow/d and 180 MJ ME/cow/d respectively). The proportion of perennial ryegrass-white clover was greater for cows on the spatial separation treatment. There was a greater lactose concentration in the milk of cows grazing spatially separated forages compare with those grazing temporally separate forages which indicates changes in rumen fermentation and variation in the supply of volatile fatty acids. Lactose is influenced by glucose supply and hence production of gluconeogenic precursors such as propionate (Bauman *et al.* 1980). Diets high in starch or water soluble carbohydrate increase propionate and thereby glucose production. There was a higher WSC of plantain than perennial ryegrass-white clover for both treatments. Perennial ryegrass-white clover in the spatial treatment had a WSC concentration of 21.1% compared with 17.3% for perennial ryegrass-white clover in the temporal treatment. This resulted in cows consuming an additional 630 g WSC in the spatial treatment compared with the temporal treatment. In spite of lower crude protein intake for cows on the spatial treatment, these cows had a higher milk protein yield. This may be due to sufficient gluconeogenic precursors from propionate which resulted in the protecting of amino acids that could then be used for milk protein synthesis (Dewhurst *et al.* 2000). Alternatively, a more synchronous supply of energy and protein in the rumen on the spatial separation treatment may have contributed to increased milk protein for cows on spatially allocated forages (Rooke *et al.* 1987; Dewhurst *et al.* 2000). Although the mechanisms behind the improved milk supply are not clear, the effect of allowing cows to select from spatially separate plantain and perennial ryegrass-white clover indicates there are greater production benefits than those from offering the same plant species consecutively in a temporal separation when offered at the same daily herbage allowance.

6.4.2 N partitioning

Allocating 100% of herbage allowance as perennial ryegrass-white clover in the afternoon has been suggested as a means of improving the efficiency of N utilisation due to improved balance of WSC and CP (Hoekstra *et al.* 2007; Gregorini *et al.* 2010). In this experiment where two major diet constituents of perennial ryegrass-white clover and plantain were allocated as spatially and temporally separated forages, N efficiency indicators; MUN and urine N concentration were not affected by timing of allocation. Perennial ryegrass-white clover offered in the afternoon to cows grazing temporally separated forages had a lower WSC concentration than perennial ryegrass-white clover offered in the morning on the spatial treatment which is inconsistent with previous experiments (Miller *et al.* 2001; Rasmussen *et al.* 2009) but indicative of differences between treatment paddocks. Of note was greater pre-grazing DM pasture mass for spatially separated perennial ryegrass-white clover. This helps to explain the difference in WSC concentration between the perennial ryegrass-white clover pasture as WSC concentration increases with leaf appearance due to accumulation of sugars through photosynthesis as shown by Fulkerson *et al.* (2001). Therefore a higher pre-grazing DM (as seen in the temporal treatment) would have accumulated more sugars.

Urine N concentration was similar between treatments averaging 2.8% N. This value is similar to that found in Chapter 3 for late lactation cows grazing a diet of 50% perennial ryegrass-white clover and 50% plantain (3.6%) or pure plantain (2.4%) but lower that pure perennial ryegrass-white clover (5.4%). Previous studies have shown the excretion of N in urine to be linearly related to N intake (Tas *et al.* 2006b; Higgs *et al.* 2012). In the current experiment N intake was 500 g/cow/d greater for cows grazing temporally separated forages. However, this did not lead to a difference in urine N concentration. It is possible that the difference in the N intake was not great enough to have in an impact on urinary N concentration. Interestingly, cows grazing spatially separated forages had greater milk N output than those grazing temporally separated forages despite a lower N intake which led to a greater NUE. This may have been due to the higher WSC concentration of the perennial ryegrass-white clover portion of the diet for cows grazing spatially separated forages. The increase WSC concentration and decreased CP from spatially separated forages led to a WSC:CP ratio of 1.4 compared with 0.9 for temporally separated forages. Edwards *et al.* (2007) suggested a WSC:CP ratio of above 0.7 may lead to increased NUE in grazing dairy cows.

A review by Selbie *et al.* (2015) found the average urinary N concentration of dairy cows in New Zealand is 6.9 g N/L. Urinary N concentrations in this experiment were over 50% lower than that average. This was due to the inclusion of plantain in the animals' diet regardless of allocation method. Plantains ability to reduce urinary N concentration compared with perennial ryegrass-white clover despite similar N intakes may be due to its mineral and secondary plant compound herbage composition. Daily urination volume is influenced mainly by water intake, which is related to the mineral load ingested and excreted by the animal. In this experiment, with the exception of magnesium, all minerals were in greater concentrations in plantain compared with perennial ryegrass-white clover. Further the concentration of sodium, a known diuretic (Ledgard *et al.* 2015), was over 50% greater for plantain than perennial ryegrass-white clover, which may have contributed to greater urine volumes thereby diluting the urinary N concentration for animals in this experiment.

The mean urine frequency and volume for each cow (2.74-2.92 L/urination and 13.4-13.5 urinations/day) are within the ranges described for dairy cattle by Selbie *et al.* (2015) in a review of urination behaviour. Similar to Edwards *et al.* (2015), Farrell *et al.* (2016) and Ravera *et al.* (2015) urine observations in this study were highly variable in volume and frequency. Urination volumes ranged from 0.5 to 9 L per event. Of note, there were no differences in urine volume output or frequency between the two treatments. Urine volume and frequency is typically related to water intake, forage composition and the presence of secondary plant compounds (Stewart 1996; Selbie *et al.* 2015). While water intake from the trough was not measured, there was no difference in water intake from herbage between treatment groups. Water intake from herbage was calculated as 68.3 L/cow/d both treatment groups with 65% coming from plantain.

Urination events for both treatments had the greatest volumes during the night, which has also been found by Misselbrooka *et al.* (2016). All urination events of above 5 L in this experiment occurred between 2100 and 0430h. Unlike Misselbrooka *et al.* (2016) where a peak in urine output occurred in early morning, urinations peaked around midnight in this study. This suggests there is a greater potential for nitrate leaching where regular night time camping areas are used, particularly where urine patches overlap.

6.5 Conclusions

- Cows grazing spatially separate forages had greater milk production than those grazing temporally separate forages
    - When offering a diet of 50% plantain - 50% pasture, this experiment suggested allocation for greatest milk production is best achieved through spatial separation.
- Urinary N concentration and urinary N output was similar between treatments. This urinary N concentration was lower than would typically be expected from cows grazing perennial ryegrass-white clover pasture alone.
    - Diets which include 50% plantain as a grazed forage type can reduce the risk of nitrate leaching by lowering urinary N output.
    - This may improve the sustainability of grazing dairy systems whilst maintaining production.

Chapter 7

Seasonal and diurnal change in the secondary metabolite composition of plantain over two growing seasons with high and low N fertiliser inputs

7.1 Introduction

Offering plantain as a pure sward alone or in conjunction with perennial ryegrass-white clover to grazing dairy cows has resulted in a large reduction in urinary nitrogen (N) output and concentration as shown in Chapter 3 and Chapter 6. The reduction in urinary N concentration appeared to be, in part, due to an increase in urine volume thereby diluting N. This may be due to the diuretic properties of plantain. Plantain is known to have high levels of secondary plant compounds which are known to have diuretic and antimicrobial properties (Rumball *et al.* 1997). Secondary metabolites are compounds produced by plants that do not act towards primary growth or development, but can contribute to plant fitness through antimicrobial and allelopathic activity (Bourgaud *et al.* 2001). Plantain is known to have 3 main secondary plant compounds; aucubin, catalpol and acteoside (Tamura *et al.* 2002).

According to Stewart (1996) plantain contains around 41 mg/g DM aucubin in the leaves. Aucubin, an iridoid glycoside, stimulates both the removal of uric acid from tissues to blood and the excretion of uric acid from the kidneys. Aucubin may be able to reduce nitrous oxide (N_2O) emissions due to its antimicrobial properties (Gardiner *et al.* 2016). Soils under plantain had lower nitrate (NO_3^-) concentration, mineralisation and nitrification rates compared with soils under other plant species, likely due to aucubins nitrification inhibiting behaviours (Massaccesi *et al.* 2015). Plantain herbage also contains the iridoid glycoside, catalpol at 0.7-5.0% of the DM (Tamura *et al.* 2002). Catalpol is the active diuretic compound of the fruit of *Catalpa ovata*. Therefore, catalpol may be contributing to increased urine volumes from animals consuming plantain (Chapter 3; Edwards *et al*. 2015; O'Connell *et al*. 2016). However, there are limited studies which quantify the concentration of catalpol in plantain herbage.

Acteoside is a phenylpropanoid glycoside that can be present in high levels within the leaves of plantain (Fajer *et al.* 1992). Similar to aucubin, acteoside is thought to have developed in plants as a defence mechanism against predation from insects. Acteoside is known for its antimicrobial (Andary *et al.* 1982; Chen *et al.* 2012), and antioxidant effects (Zhou *et al.* 1991; Chen *et al.* 2012). Acteoside can be found in differing concentrations depending on the time of year that plantain is harvested. Spring harvested plantain contained concentrations of acteoside at 15 mg/g DM which increased to 41 mg/g DM in autumn (Tamura *et al.* 2002).

A review by Gardiner *et al.* (2016) concluded there is considerable potential for nitrification inhibition if ruminants consume these naturally occurring inhibitory metabolites in their forage and excrete them in urine, which would then directly apply these inhibitory compounds to urine patches. Navarrete *et al.* (2016) also showed beneficial effects of secondary plantain metabolites on ruminants, with plantain producing 40% less rumen ammonia (NH_3) than chicory. Therefore to reduce the environmental impacts of a New Zealand grazing systems, management strategies which enhance the concentration of aucubin, catalpol and acteoside in plantain would be beneficial. There is currently little information which quantifies the concentration of secondary metabolites in plantain grown in New Zealand. This chapter aimed to examine the effects of N fertiliser inputs and time of day on the concentration of secondary plant compounds in Ceres Tonic plantain. The experiment also aimed to characterise the seasonal change in the secondary plant compounds in the two growing seasons following establishment of plantain.

7.2 Materials and methods

7.2.1 Experimental site and design

The experiment was conducted over two growing seasons from 29 October 2014 to 31 March 2016 at the Lincoln University Research Dairy Farm, Canterbury New Zealand (43^{0}64’S, 172^{0}46’E). A split plot design was used with 3 replicates. Treatments were N rate (180 and 450 kg N/ha/year) and harvest time (am (0700 h) and pm (1600 h)). The experiment used the plantain plots which were sown as a larger experiment. The area used is described in Chapter 4. Briefly, plantain was sown in large plots in a randomised block design. Nitrogen rate was randomly assigned as a split plot treatment.

N fertiliser was applied following each defoliation as calcium ammonium nitrate (27 : 0 : 0 : 0; N : P : K : S), with the total annual N application rate split evenly throughout the year at rates of 180 kg N/ha/year for the low N inputs treatment and 450 kg N/ha/year for high N input treatment.

7.2.2 Management

Management of the experimental area in the first year is previously described in Chapter 4, Section 4.2.2. Briefly, the plots were managed under a cut and carry regime. Plantain was harvested approximately every 25 days. Harvest dates are presented in Table A. 6.

In year two of the experiment herbicide T Max (active ingredient aminopyralid) was applied at 40 mL/10 L water on the 7 October 2015.

7.2.3 Meterological data

Rainfall and air temperatures for the experimental period are presented in Figure 7.1. Total annual rainfall during the first year of experiment (386 mm) was lower than the average long term rainfall of the last 30 years (599 mm). This shortfall in rain was compensated with irrigation in the key growing season, which was applied at a rate of 23 mm/week, supplemental to rainfall, between November and March. In the second year rainfall was varied, with around two time greater rainfall than the average occurring in February and June. In spring and autumn of year 2, rainfall fell below the long-term average. This was compensated with irrigation which was applied at a rate of approximately 30 mm/week when rainfall was insufficient.

The monthly air temperatures (Figure 4.1b) showed a similar trend to the long-term average air temperature.

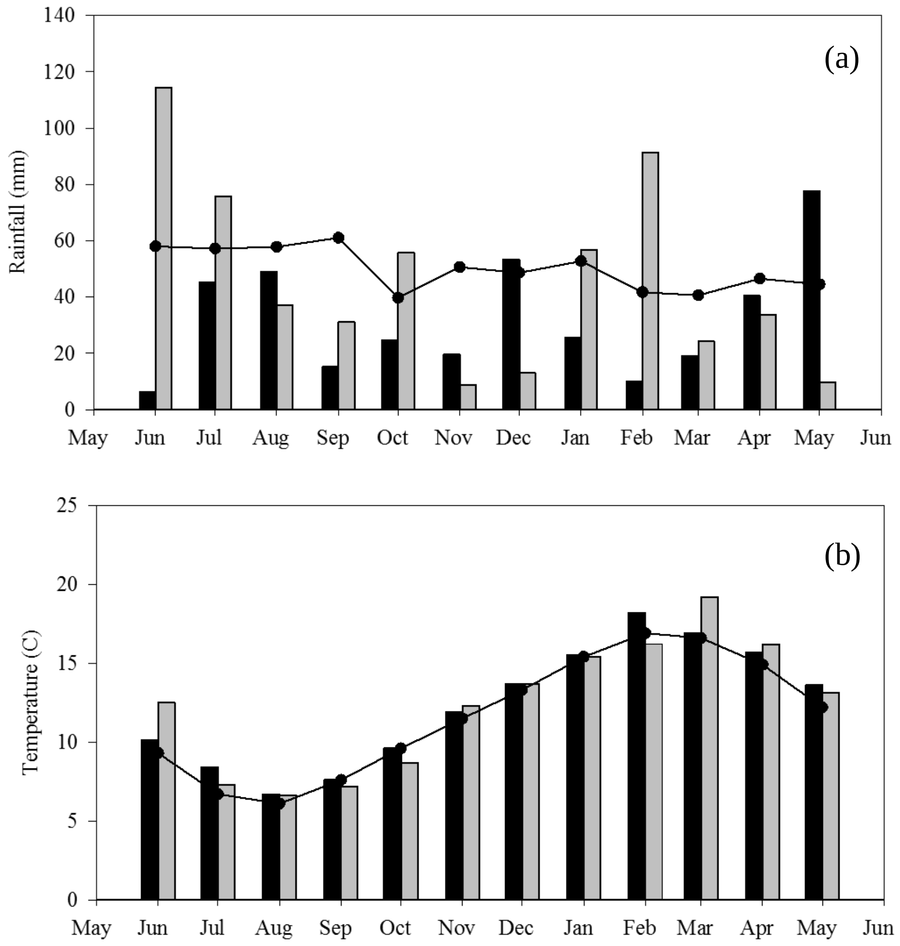


Figure 7.1 (a) Monthly mean monthly rainfall and (b) mean air temperature for June 2014–May 2015 (year 1) and June 2015-May 206 (year 2) at Lincoln, Canterbury, New Zealand. Data were collected from Broadfields Meteorological Station, 1 km from research site. June 2014–May 2015 (■), June 2015-May 2016 (□), long-term average (1981–2010) (——).

7.2.4 Herbage measurements

At each harvest, samples were taken from each plot at 0700 h and 1600 h. A 1 m strip was cut at grazing height (4 cm) using electric hand shears. Each sample was frozen and freeze dried before being ground through a 1 mm sieve using a M200 rotor mill (Retsch Inc. Newtown, Pennsylvania, USA). Ground material was analysed for secondary plant compounds (catalpol, aucubin and acteoside) using high performance liquid chromatography (HPLC) by Massey University analytical laboratory following procedures of Tamura *et al.* (2002).

In the first year, harvests in spring occurred between 9 October 2014 and 30 October 2014. Summer harvests occurred between 24 November 2014 and 10 February 2015 and autumn harvests between 10 March 2014 and 18 May 2015. In year two harvests in spring occurred between 9 October 2014 and 30 October 2014. Summer harvests occurred between 24 November 2014 and 10 February 2015 and autumn harvests between 10 March 2014 and 18 May 2015.

7.2.5 Statistical analysis

Data were analysed by general ANOVA using Genstat (VSN International LTD. 2102) with replicates as random effect and year, season, N rate and time of day as fixed effect. Differences were considered significant at $P<0.05$.

7.3 Results

7.3.1 Aucubin

The concentration of aucubin in plantain was highest in spring of year 1 (P<0.001) (Table 7.1). The concentration of aucubin in plantain was not affected by N fertiliser inputs or time of day (P>0.050). Plantain herbage samples had a greater (P<0.001) concentration of aucubin in spring of the first year of growth (average 2.18 mg/g DM) compared with year 2 (average 1.89 mg/g DM). In summer the concentration of aucubin in plantain was 1.01 mg/g DM in year 1 which reduced (P=0.011) to 0.69 mg/g DM in year 2. In autumn there was no difference (P=0.172) in the average aucubin concentration between year 1 and 2. Aucubin concentration was lowest in summer.

Table 7.1 Mean herbage aucubin concentration (mg/g DM) of plantain harvested in year 1 (2014/15) or year 2 (2015/16) after establishment at 450 kg N/ha/yr (high) and 180 kg N/ha/yr (low) fertiliser inputs harvested either in the morning (AM) or afternoon (PM).

Year	N inputs	Time	Spring	Summer	Autumn
1	Low	AM	5.85	1.28	2.42
		PM	3.86	0.899	1.73
	High	AM	3.93	1.07	2.52
		PM	3.20	1.72	2.23
2	Low	AM	2.13	0.708	2.34
		PM	1.66	0.922	2.97
	High	AM	2.77	0.574	2.31
		PM	1.68	0.566	3.68
SEM			0.172	0.146	0.155
Year				0.001	
Season				<0.001	
N rate				0.797	
Time				0.243	
Year*Season				<0.001	
Year * N rate				0.368	
Year * time				0.100	
Season* N rate				0.286	
Season* time				0.022	
Year*season*N rate*time				0.527	

7.3.2 Acteoside

The concentration of acteoside in plantain herbage was greatest (P<0.001) in spring. This seasonal effect was consistent (P=0.371) between sampling years. In summer and autumn N inputs and time of day had no effect on the concentration of acteoside in plantain (Table 7.2). In spring the concentration of acteoside was lower (P<0.001) at high N fertiliser inputs. This N fertiliser effect was greater (P=0.050) in year 1 than year 2. There was a time of day effect in spring only. In spring, plantain herbage harvested in the morning had a higher (P<0.001) concentration of acteoside than herbage harvested in the afternoon. This diurnal effect was greater (P=0.019) for plantain with low N fertiliser inputs.

Table 7.2 Mean herbage acteoside concentration (mg/g DM) of plantain harvested in year 1 (2014/15) or year 2 (2015/16) after establishment at 450 kg N/ha/yr (high) and 180 kg N/ha/yr (low) fertiliser inputs harvested either in the morning (AM) or afternoon (PM).

Year	N inputs	Time	Spring	Summer	Autumn
1	Low	AM	36.0	5.86	10.2
		PM	14.5	1.77	8.86
	High	AM	16.7	2.32	8.08
		PM	9.08	4.53	6.49
2	Low	AM	22.9	3.36	4.97
		PM	10.6	6.44	7.49
	High	AM	19.4	2.23	4.42
		PM	10.6	4.07	8.31
SEM			1.02	0.863	0.834
Year				0.102	
Season				<0.001	
N rate				0.005	
Time				0.001	
Year*Season				0.378	
Year * N rate				0.050	
Year * time				0.054	
Season* N rate				0.026	
Season* time				<0.001	
Year*season*N rate*time				0.420	

7.3.3 Catalpol

Catalpol was consistently in concentrations lower than 0.4 mg/kg DM (Table 7.3). The concentration of catalpol was greatest (P<0.001) in spring and summer of year 1. There was no seasonal effect on the concentration of catalpol in year 2. In spring and autumn there was no effect of N fertiliser or harvest time on the concentration of catalpol. The concentration of catalpol in plantain herbage was reduced (P<0.001) by approximately 90% from year 1 to year 2 in spring and summer. In autumn the opposite was seen, the concentration of catalpol was two times greater (P=0.007) in year 2 than in year 1. In spring the catalpol concentration increased (P<0.001) from 0.004 to 0.012 mg/g DM with increasing N fertiliser inputs in the morning.

Table 7.3 Mean herbage catalpol concentration (mg/g DM) of plantain harvested in year 1 (2014/15) or year 2 (2015/16) after establishment at 450 kg N/ha/yr (high) and 180 kg N/ha/yr (low) fertiliser inputs harvested either in the morning (AM) or afternoon (PM).

Year	N inputs	Time	Spring	Summer	Autumn
1	Low	AM	0.286	0.205	0.003
		PM	0.326	0.118	0.006
	High	AM	0.257	0.116	0.005
		PM	0.108	0.390	0.006
2	Low	AM	0.022	0.012	0.014
		PM	0.015	0.042	0.011
	High	AM	0.013	0.013	0.012
		PM	0.043	0.023	0.011
SEM			0.014	0.012	0.012
Year				<0.001	
Season				<0.001	
N rate				0.638	
Time				0.424	
Year*Season				<0.001	
Year * N rate				0.679	
Year * time				0.937	
Season* N rate				0.012	
Season* time				0.060	
Year*season*N rate*time				<0.001	

7.4 Discussion

This experiment aimed to characterise the concentration of three secondary plant compounds in plantain herbage across two growing seasons. The effects of N inputs and time of day on the concentration of metabolites were also examined.

Aucubin may be useful for reducing N_2O, and NO_3^- losses from urine patches (Gardiner *et al.* 2016), due to its antimicrobial properties (Dietz *et al.* 2013). This study confirms plantain contains aucubin, with an average concentration of 2.01 mg/g DM. By applying 12 mg of pure aucubin to soil samples, Dietz *et al.* (2013) observed an almost complete inhibition of NO_3- production. For a grazing animal consuming plantain, it is possible aucubin would be excreted in the urine (Keir *et al.* 2001), thereby applying aucubin directly to the soil which may be able to reduce N losses at the urine patch. Whether or not the concentration observed in the current study is large enough to have any meaningful effect on reducing N losses or increasing urine volume is unclear and further research is required. Gardiner *et al.* (2016) reviewed the effects of aucubin on N losses from agricultural systems. The authors did not note any diuretic effect of aucubin. However this has not yet been tested and may require a dose response experiment to determine whether aucubin has any influence on the urine output of dairy cows.

Overall acteoside was present in plantain in the greatest concentrations, of up to 36.0 mg/g DM, for all seasons, regardless of treatment. Acteoside is thought to have developed in plants as a defence mechanism against predation from insects (Fajer *et al.* 1992). The compound is known for its antimicrobial and antioxidant effects. There are no known negative effects of acteoside, therefore, increased concentrations which exist in plantain may be favourable for grazing animals. The microbial properties of acteoside, which are similar to those in aucubin, may also be useful for reducing N losses from a urine patch by inhibiting NO_3- production (Gardiner *et al.* 2016). Further research is required to confirm this hypothesis.

Catalpol was only present in very low in concentrations (<0.390 mg/g DM). Catalpol is known for its diuretic properties (Deaker *et al.* 1994; Tamura *et al.* 2002). However, at the concentrations observed in this study, the compound is unlikely to have any effect on the grazing animal. Zhu *et al.* (2016) found diuretic properties were seen when rats were dosed with 50 mg catalpol/kg body weight. In the current experiment, the maximum catalpol concentration observed was 0.390 mg/g DM. For a 500 kg dairy cow consuming 18 kg DM/day of plantain, this would only equate to a maximum intake of 14 mg catalpol/kg LWT, which may not be a large enough concentration to cause diuresis. However the critical level required to have an effect on a dairy cow has not yet been determined.

7.4.1 Seasonal changes

All of the secondary metabolites showed seasonal differences in their concentrations. Aucubin and acteoside had a similar seasonal distribution. The concentration of aucubin and acteoside appeared to peak in the first spring and was consistently lowest in summer. However, aucubin showed an annual change in concentration, with a lower amounts present in spring of year 2. For acteoside, there was no change in the seasonal concentration from year 1 to year 2. This may suggest that the seasonal influence on the presence of acteoside remains consistent after the first year of growth.

Catalpol concentration peaked in the first spring and summer of growth and decline to very low concentration following summer of year 1 (<0.05 mg/g DM). At this low concentration, it is unlikely for catalpol to have any effect on the grazing animal (Zhu *et al.* 2016). This suggests that despite catalpol being the only compound with recognised diuretic properties (Deaker *et al.* 1994; Tamura *et al.* 2002), the increase in urine volume for cows grazing plantain in Chapter 3 may not be due to catalpol. However the critical level of catalpol required to have an effect on a dairy cow has not yet been determined.

Bowers *et al.* (1992) reported an increase in iridoid glycoside content over the growing season for plantain. In their experiment aucubin peaked in autumn with a concentration of around 40 mg/g DM. This concentration was up to 20 times greater than seen in the current experiment. Similar to Bowers *et al.* (1992), Tamura *et al.* (2002) reported much higher secondary metabolite concentrations compared with the current experiment. In their experiment, spring harvested plantain contained concentrations of acteoside at 15 mg/g DM which increased to 41 mg/g DM in autumn. The inconsistencies in secondary metabolite concentrations seen in the current experiment and those reported elsewhere may have been due to environmental differences as the experiment by Tamura *et al.* (2002) took place in Japan and the experiment by Bowers *et al.* (1992) was conducted in America. The presence of secondary plant metabolites may be linked to external factors. Air temperature, solar radiation, and nutrient availability are considered to be major factors in influencing plant secondary metabolite abundance (Tamura *et al.* 2002).

The results from the current study also differed from those presented in Chapter 3 and Chapter 6. Chapter 3 found greater concentrations of secondary metabolites in autumn compare with spring. The concentration of secondary metabolites tended to increase in the second year of growth as measured in Chapter 6 (which used the same paddocks and cultivar (tonic) as Chapter 3). This may be attributed to difference in pasture mass at the time of harvest as the plantain paddocks tended to have a greater mass than the plots, although this was not measured. The inconsistencies in secondary metabolite concentrations within the same cultivar of plantain, suggest further research is required to understand the mechanisms which drive metabolite production. What was consistent across all chapters, was the ratio to which each secondary metabolite was present in relation to each other. Acteoside consistently had the greatest concentration, aucubin intermediate and catalpol lowest.

7.4.2 N input effects

The results of this experiment suggests that N fertiliser inputs have little influence on the presence of secondary metabolites. There was a small, inconsistent N fertiliser influence on the concentration of catalpol in summer. However, regardless of the N fertiliser influence, the concentration of catalpol remained very low (<0.390 mg/g DM). There was a negative effect of N fertiliser on the concentration of acteoside, but this was only seen in spring of year 1. This negative relationship between N fertiliser and secondary plant metabolites in plantain has been reported previously. Tamura (2001) showed the concentration of aucubin and acteoside were lower in plantain with additions of N compared with those which received no N fertiliser.

7.4.3 Time of day effects

There was little to no effect of time of day on secondary metabolite concentration. The concentration of aucubin was unaffected by harvest time. This suggests management strategies (N fertiliser or harvest time) cannot be used to alter the concentration of aucubin in plantain herbage. Also following the same trend seen for N fertiliser, an inconsistent time of day effect on catalpol was seen for summer harvested plantain only. Again this diurnal change was not useful to increase the catalpol concentration.

There was a strong time of day effect on the concentration of acteoside in spring only. While the concentration of acteoside in plantain was greater in the first year, there was no difference in the effects of time of day between the first and second year of growth in spring. This suggests that, at least in the first year of growth, a diurnal reduction in acteoside can be expected in spring. The mechanisms behind a diurnal decrease in acteoside are unclear. The result seems counterintuitive to previous research. Tamura (2001) found that the concentration of acteoside was lower for plantain grown in the shade than for plantain in sunlight. They concluded that shade represses the growth and accumulation of aucubin and acteoside in plantain. Therefore it was assumed plantain harvested in the morning would have had a lower acteoside concentration than that harvested in the afternoon which was not the case in this experiment.

7.5 Conclusions

- The concentration of catalpol in plantain was very low (<0.390 mg/g DM) throughout the experiment. At these low concentrations catalpol is unlikely to cause an effect on a grazing animals.

- The concentration of acteoside was the greatest of the secondary metabolites and appeared to show a seasonal pattern with concentrations peaking in spring and declining in summer.

- The reasons for differences in metabolite concentrations presented in this experiment and those seen in previous experiments are unclear and suggest further research is required to understand the mechanisms driving secondary metabolite production in plantain.

- Secondary plant metabolites could not be increased by altering N fertiliser inputs or by harvesting in the afternoon.

Chapter 8

General Discussion

8.1 Summary and justification of this study

Regional Councils throughout New Zealand have been developing regulations that place a limit on the amount of nitrogen (N) leaching from agricultural land. The predominant source of N leaching from dairy farms is the urine patch. This is because feed, in the form of perennial ryegrass-white clover pasture, supplies the animal with excess N which is subsequently excreted, predominately in the urine (Di *et al.* 2002a). Urine deposited N is at rates (up to 800 kg N/ha) which far exceed plantain uptake. Excess N is readily converted to nitrate (NO_{3-}) which is easily leached when drainage occurs. Despite difficulties in managing N in pasture based systems, this thesis recognises the use of forages as a low cost, competitive advantage of New Zealand dairy systems. Therefore the main objective of this study was to identify an alternative forage or management strategy which reduce urinary N losses from grazing dairy cows without impeding production.

This thesis reports a series of experiments conducted in the field to evaluate milk production, urine composition and urine output from cows grazing plantain, to determine the effects of diurnal and N fertiliser management strategies on herbage N, nutritive value and rumen degradation characteristics of alternative forages and to characterise secondary plant compounds in plantain. These results were presented and discussed in detail in chapters 3 to 7. In brief, Chapter 3 evaluated the milk production, urine composition and urine output from cows grazing plantain and perennial ryegrass-white clover in early and late lactation; Chapter 4 compared the effect of N fertiliser and time of day on the nutritive value of alternative forages; Chapter 5 determined the rumen degradation characteristics of plantain and perennial ryegrass-white clover harvested in the morning and afternoon; Chapter 6 compared allocation methods (as determined by Chapter 4 and Chapter 5) of 50% perennial ryegrass-white clover and 50%; and Chapter 7 determined the concentration of bioactive compounds, catalpol, aucubin and acteoside in plantain herbage across two growing seasons and the effects on N fertiliser and time of day on the concentrations of metabolites. This overall discussion chapter presents an integrated discussion of the key findings in the experimental chapters and will focus on areas for further research to better understand the mechanisms driving a reduction in urinary N output and the on farm implications of this.

8.2 Main findings of this research

Previous studies have demonstrated that cows grazing ‘diverse’ pastures containing herbs (plantain and chicory) excrete urine which has lower N concentrations compared with cows grazing perennial ryegrass-white clover pastures (Woodward *et al.* 2012; Totty *et al.* 2013; Edwards *et al.* 2015; Bryant *et al.* 2017). This study focussed specifically on feeding plantain as a strategy to reduce N excretion. In Chapter 3, milk production from cows grazing plantain or 50% perennial ryegrass-white clover and 50% plantain was greater than milk production from cows grazing perennial ryegrass-white clover in late lactation and similar in early lactation. However, feeding plantain at 50 or 100% of the diet resulted in significant reductions in both urine N concentration and urine N excretion.

Chapter 4 found a greater diurnal effect on the chemical composition of perennial ryegrass compared to plantain. The accumulation of sugars diurnally was greater for perennial ryegrass than plantain which led to an improved water soluble carbohydrate (WSC) to crude protein (CP) ratio when perennial ryegrass is harvested in the afternoon. The greater WSC:CP ratio in perennial ryegrass harvested in the afternoon may be beneficial to improve nitrogen use efficiency (NUE) (Edwards *et al.* 2007). Further, Chapter 5 found that harvesting in the afternoon had larger effect on improving the effective degradability for perennial ryegrass-white clover than plantain. The diurnal improvement in effective degradability of perennial ryegrass-white clover was attributed to a diurnal reduction in fibre. These results suggest when including pure swards of plantain in a system, management strategies which allow for allocation of perennial ryegrass-white clover in the afternoon and plantain in the morning may provide the most benefits in terms of improving milk production while reducing urinary N. This hypothesis was tested in Chapter 6.

In Chapter 6 cows grazing a spatially separate diet of 50% perennial ryegrass-white clover and 50% plantain had greater milk production than those grazing temporally perennial ryegrass-white clover. Allocation method had no effect on the urinary N concentration and total urine output. Urinary N output and concentration from cows in this experiment was lower than what would be expected from cows grazing perennial ryegrass-white clover alone due to the inclusion of plantain in the animals’ diet.

Chapter 7 found the concentration of secondary plant metabolites was varied. Acteoside consistently had the greatest concentration, aucubin intermediate and catalpol was in concentrations lower than would be required to have any effect on the grazing animals. The concentration of metabolites could not be altered by N fertiliser and typically did not show diurnal fluctuations.

8.3 Drivers of reduced urinary N from plantain

8.3.1 N intake

A feature of the results from the grazing experiments of this thesis was the reduction in urinary N concentration when cows were offered plantain at either 100% or 50% of their diet compared with 100% perennial ryegrass-white clover diets. Previous experiments which have compared urinary N from cows grazing perennial ryegrass-white clover and alternative forages have attributed any reduction in urinary N to a reduction in N intake (Tas *et al.* 2006b; Woodward *et al.* 2012; Totty *et al.* 2013). A linear relationship between N intake and urinary N output has been well described (Tas *et al.* 2006b). In this thesis cows grazing 100% or 50% plantain were consuming at least as much N as those grazing 100% perennial ryegrass-white clover. Data was compiled from this experiment and other grazing experiments where plantain was included in the diet of dairy cows as either a pure sward which comprised at least 20% of the animals diet (Chapter 3, Chapter 6, Cheng *et al.*, 2017, Nkomboni, 2017, Minnee *et al,* 2017) or in a mix as less than 20% of the diet (Woodward *et al.* 2012; Totty *et al.* 2013; Edwards *et al.* 2015; Bryant *et al.* 2017). There was no relationship between N intake and urinary N concentration (P=0.524; r^2=0.0321) for those experiment which fed animals pure swards of plantain, and the mean of urinary N was lower than for animals grazing perennial ryegrass-white clover (Figure 8.1a). Further for those experiments which measured urine output (volume excreted per day), a positive relationship between N intake and urinary N output was observed for cows grazing perennial ryegrass-white clover, this relationship was different (P<0.001) for cows grazing plantain (Figure 8.1b). This suggests a reduction in urinary N concentration when plantain is included in the diet of dairy cows is not driven by N intake like it is for perennial ryegrass-white clover.

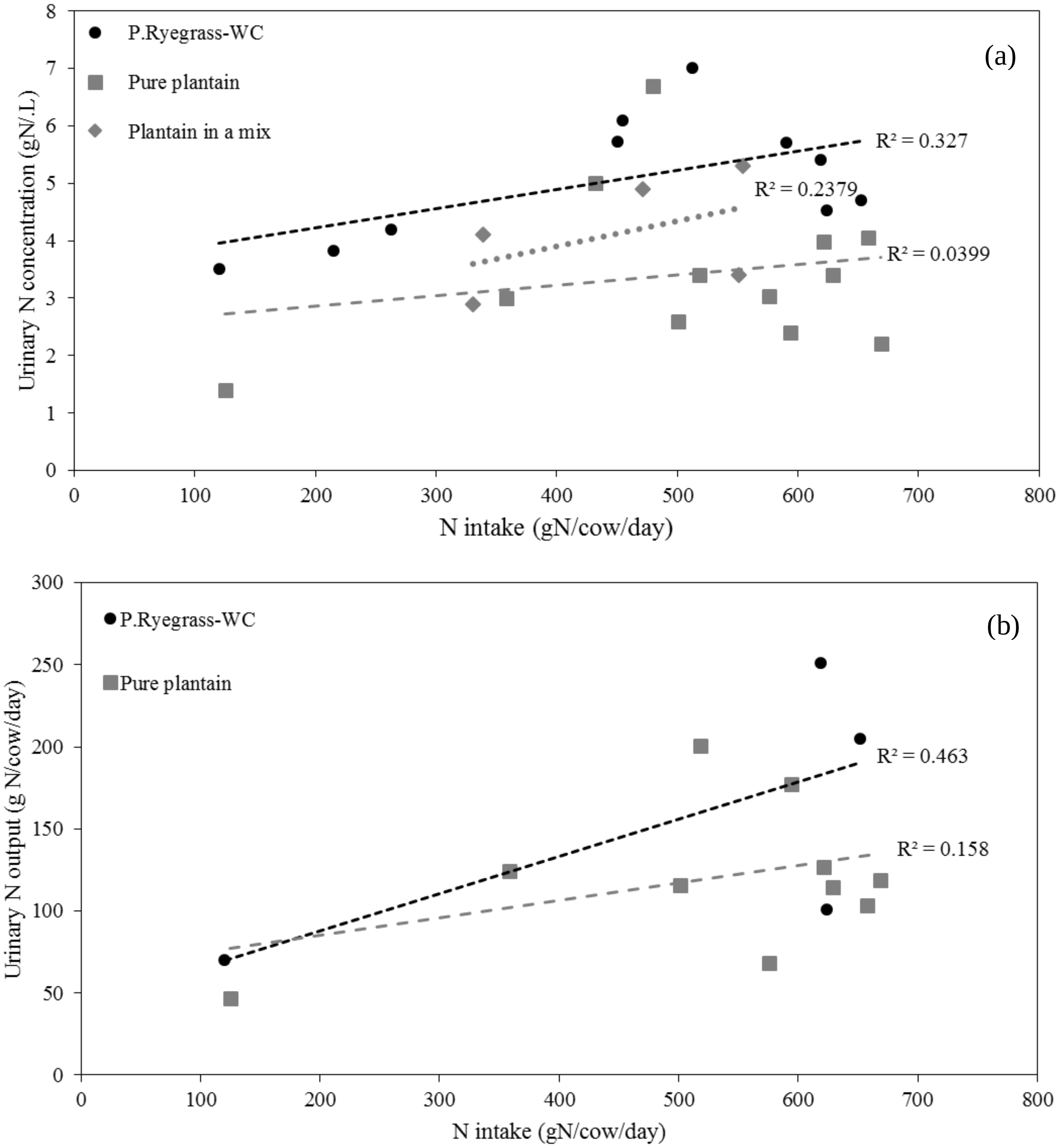


Figure 8.1 Relationship between N intake (g N/cow/day) and the N concentration of urine (g N/L) (a) and urinary N output (g N/cow/day) (b) for cows grazing plantain as a monoculture (■ --) or in a diverse mix (♦. . .) or perennial ryegrass-white clover (●---).

The exact mechanisms driving the reduction in urinary N output for cows grazing plantain and perennial ryegrass-white clover are unclear. A route for increased N output, was thought to be via an increase in urine volume. An increase in urine volume partially explained the reduction in urinary N via dilution. However, this increase in urine volume was not large enough to offset the large reduction in urinary N concentration, therefore, overall urinary N output was reduced from cows grazing plantain. It is important to note that the urinary N output in this study was estimated based on spot samples of urine taken following afternoon milking and the following morning milking. Betteridge *et al.* (2013) showed a variation in the composition of urine from cows throughout the day. Therefore in this study total N output may vary from the calculated estimation. However, size of the reduction in urinary N for cows grazing 100 or 50% plantain in this study suggests that even with a variation in diurnal urine composition, a reduction in urinary N output would be observed.

One route which N partitioning may have been altered is via the faeces. There was no difference in faecal N concentration between the diets in this study. However total faecal output was not measured. There may have been an increase in the amount of dung excreted by cows grazing plantain and 50-50 perennial ryegrass-white clover-plantain compared with those on perennial ryegrass-white clover alone. To confirm exact N losses from cows grazing plantain, further research which captures all of the excreta is required.

8.3.2 Mineral concentration effect

The mineral composition and concentration of forages can influence urinary output. In this study plantain consistently had a greater mineral load than perennial ryegrass-white clover. The concentration of sodium, a known diuretic (Ledgard *et al.* 2015), was greater for plantain compared with perennial ryegrass-white clover. The main effect of sodium is to increase water intake and therefore urine output, causing a water diuresis. Water intake was measure in the early lactation experiment of Chapter 3. In the experiment water intake from the trough was lowest for cows grazing plantain (10.0 L/cow/day) intermediate from cows on 50-50 plantain-pasture (22.8 L/cow/day) and greatest from cows on perennial ryegrass-white clover (40.3 L/cow/day). However, the low DM% of plantain (14.5 %) resulted in total water intake (water from trough and feed) being greatest from plantain (163 L/cow/day) and similar between 50-50 pasture-plantain (140 L/cow/day) and perennial ryegrass-white clover (147 L/cow/day). This result indicates that in this experiment, sodium did not appear to have a large influence on water intake.

8.3.3 Water diuresis

An increase in urine volume from animals grazing plantain can reduce the concentration of N in the urine via dilution. Urine volume is closely related to water intake. The majority of a cow’s water intake comes from feed rather than water consumed. Typically forages have a DM% ranging from 15-20%. Therefore, forages with a lower DM% generally increase water intake leading to increased urine volume. In this study plantain had an average DM of 12.0% compared with 17.3% for perennial ryegrass-white clover. This was also seen by Cheng *et al.* (2017) who measured water intake and urination volumes for stall fed heifers. The authors found animals fed plantain had greater total water intake (34 L/cow/d) than those fed perennial ryegrass-white clover (25.4 L/cow/d) and attributed this difference to the lower DM of plantain. For heifers on plantain, 86.3% of their total water intake came from forage compared with 70.3% for those fed perennial ryegrass-white clover. Animals fed plantain had ~50% lower urinary N concentration and ~30% lower urinary N output compared with heifers fed perennial ryegrass-white clover. These results are similar to those present in the current study. However, the increase in water intake when grazing plantain may not fully explain an increase in urine volume.

An experiment which matched water intake across forages found sheep fed plantain excreted over 5 L/day/sheep compared with 4 L/day/sheep from sheep grazing perennial ryegrass (Judson, G., unpublished data). Further, data collected in a metabolism stall experiment strongly suggests a sustained diuretic effect when plantain was fed to sheep (O'Connell *et al.* 2016). Though not measured, O'Connell *et al.* (2016) suggested that diuresis may have been due to bioactive compounds known to exist in the leaves of plantain.

8.3.4 Seondary metabolite effect

Previous experiments have suggested that plant secondary compounds in plantain herbage may be contributing a reduction in urinary N concentration, predominantly via dilution. Of the secondary metabolites, catalpol has been identified as a diuretic. In this study catalpol was never present in any meaningful concentrations. Therefore, the reduction in urinary N for cows grazing plantain is unlikely to be attributed to catalpol. However, the critical concentration of catalpol required to cause diuresis in dairy cows is unknown and requires further investigation. Aucubin and acteoside have previously reduced NH_3 production in the rumen (Navarrete *et al.* 2016). Aucubin reduced acetate and total VFA production likely due to its bactericide activity, while acteoside increased propionate and total VFA suggesting improved rumen fermentation. Therefore, the reduction in urinary N for cows grazing plantain may be in part due to aucubin or acteoside. These compounds may also act as diuretics. Further research is required to test this theory and to determine critical levels required to reduce urinary N concentration and output.

8.4 Implications for nitrate leaching

This thesis presents a significant opportunity for plantain to be used in a grazing situation to reduce urinary N losses from dairy cows. Chapter 3 found that 100% plantain reduced urinary N concentration by over 50% from early and late lactation dairy cows compared to perennial ryegrass-white clover. Further Chapter 3 and Chapter 6 showed urinary N concentration from cows on 50% plantain was around 30% lower than from perennial ryegrass-white clover. Urinary N output was also reduced by 15% and 53% for cows grazing 100 or 50% plantain respectively in late lactation and by 44% and 42% respectively in early lactation. This was in part due to a dilution effect caused by an increase in urine volume. However, these short term small scale experiments did not capture all of the required information to understand total N losses from a farm system when plantain is used as a grazing forage. In order to allow the transfer of this knowledge to the farming situation, further research needs to be carried out. This includes understanding N leaching under plantain and from the urine of cows grazing plantain.

In a lysimeter study, plantain incorporated in a mix with Italian ryegrass and white clover, reduced N leaching from applied urine by 45% compared with N leaching from perennial ryegrass-white clover (Woods 2017). Both pasture types received the same application of urine N. The reduction in N losses through the soil were in part due to an increase in N uptake from the mix and in part due to plantains nitrification inhibiting properties (Dietz *et al.* 2013). The results from this study where plantain has shown a reduction in the urinary N concentration combined with the lower N leaching losses seen by Woods (2017) suggest overall N leaching through the soil can be massively reduced. When Woods (2017) applied urine collected from cows grazing a plantain, Italian ryegrass and white clover at a rate of 508 kg N/ha, which is similar to the calculated N loading rate from cows grazing plantain in Chapter 3, to a lysimeter with the same pasture, N leaching from the urine was reduced by 89%. This reduction in N leaching losses was in part due to an N uptake effect associated with cool season growth of plantain (Stewart 1996; Moorhead *et al.* 2009) and part due to the N loading the effect, whereby animals grazing plantain excreted less N in the urine. These results suggest there is considerable potential to reduce overall N leaching from a farm system when plantain is used as a grazed forage.

The exact mechanisms behind a nitrification inhibitory as seen by Dietz *et al.* (2013) are unclear but may be attributed to secondary plant compounds. This thesis only measured N and purine derivative (PD) excretion in the urine of cows grazing plantain. There is a potential for aucubin and acteoside to be excreted in the urine of cows grazing plantain. Previous experiments have found lower nitrate (NO_3^-) concentration, mineralisation and nitrification rates under plantain had lower compared with soils under other plant species (Massaccesi *et al.* 2015). The authors attributed the NO_3^- reduction to aucubin's nitrification inhibiting behaviours. These behaviours may also be exhibited by acteoside (Gardiner *et al.* 2016). There is a potential for animals grazing plantain to directly deposit aucubin and acteoside on the soil via urine. However further research is required to determine if aucubin and acteoside are present in the urine of animals grazing plantain, what the effects are at the urine patch and what concentration is required to achieve a reduction in NO_3^-.

The experiment by Woods (2017) measured N leaching at the urine patch only, and did not consider paddock scale. Therefore, more work is required on the number and volume of urinations across a paddock as these determine paddock N loading, degree of urine overlap and therefore risk of N leaching from a paddock. None the less, a massive increase in the number of urine patches would be required to prevent this beneficial effect of plantain reducing the risk of N leaching occurring and there is limited evidence of this.

8.5 Proportion of plantain required in the diet

An exciting result from this study was the large reduction in urinary N concentration and output from cows grazing plantain and 50-50 perennial ryegrass-white clover-plantain. However, it is possible a reduction could occur with a lower proportion of plantain in the diet. Therefore, a logical next step would be to evaluate the proportion plantain required to achieve a reduction in urinary N losses from dairy cows. Recently an experiment by Nkomboni (2017) at the Lincoln University Research Dairy Farm evaluated urinary N concentration and urine output from cows grazing plantain at varying proportions of the diet. The study confirmed that plantain has similar feed value and milk production potential to perennial ryegrass-white clover when offered as green leafy herbage to dairy cows. Further, the author found that feeding greater than 30% plantain in the diet resulted in significant reduction in both urine N concentration and urine N excretion. This 30% threshold has implications for how plantain is managed in a farm system.

8.6 Farm system application

8.6.1 Grazing management of plantain

In order to allow for at least 30% of an animals diet to be plantain, it is likely the forage will need to be fed as a monoculture. In diverse pasture mixes, plantain rarely comprises more than 15% of the DM on offer (Nobilly *et al.* 2012; Woodward *et al.* 2012; Totty *et al.* 2013; Edwards *et al.* 2015; Bryant *et al.* 2017). One strategy that may allow for the successful incorporation of pure swards of plantain into a grazing system, may involve allocating animals to plantain paddocks following every third milking. This would result in animals on average receiving at least 30% of their diet across a week as plantain. This is similar to the temporal allocation strategy used in Chapter 6.

In Chapter 6 a difference in milk production was observed between temporal and spatial allocation of perennial ryegrass-white clover and plantain. There was no difference in urinary N concentration and output which suggest the timing of allocation was not important in terms of urine composition. Milk production was reduced for cows on the temporal treatment. Cows allocated to the spatial treatment produced 1.53 kg MS/cow/day milk compared with 1.37 kg MS/cow/day from cows on the temporal treatment despite similar herbage allocation and DM intake. However, the results from this study are limited due to the differences in the perennial ryegrass-white clover component of the diet. The temporal treatment required an additional area of perennial ryegrass-white clover due to lower DM production compared with plantain. The additional area of perennial ryegrass-white clover had a greater pre-grazing mass. This influenced the chemical composition of the perennial ryegrass-white clover component. Therefore, milk production results may be limited due to the differences between treatments in the perennial ryegrass-white clover proportion of the diets. Temporal allocation of forages may prove useful for including plantain in an animals diet as a monoculture while maintain milk production. Further research which allocates animals to pure plantain following every third milking would be useful to determine if this is a feasible strategy to ensure animals received at least 30% of their diet as plantain without impeding milk production.

8.6.2 Silage

Another method to incorporate plantain into an animal’s diet is as a conserved feed. Judson *et al.* (2016) compared the urinary N concentration of dairy heifers in winter on a kale diet supplemented with either plantain or perennial ryegrass baleage. Liveweight gain was similar between heifers supplemented with perennial ryegrass baleage and those given plantain baleage. However, urinary N concentration from heifers supplemented with plantain baleage (0.36% N) was lower than those supplemented with perennial ryegrass baleage (0.53% N). While this experiment used heifers, the large reduction in urinary N for heifers supplemented with plantain, suggest that these results would also be seen for a lactating dairy cow.

8.6.3 DM production

A farmer’s decision to sow a forage is largely based around the DM production potential of the forage. Therefore, in order for plantain to be included in a grazed system it would need to produce at least as much DM as perennial ryegrass-white clover. This experiment did not measure DM production from plantain. However, previous experiments have shown that plantain, sown in a mix can produce greater DM in the summer and autumn compared with grass based pastures, which allows a greater distribution of DM yield throughout the year

(Moorhead *et al.* 2009). This is due to plantains drought tolerance and considerable summer heat tolerance (Stewart 1996; Rumball *et al.* 1997). More recent work has found that plantain as a monoculture can produce more DM annually than perennial ryegrass (Martin *et al.* 2017). In the cut and carry small plots experiment, Martin *et al.* (2017) found plantain produced 10 069 kg DM/ha/year compared with 7702 kg DM/ha/year from perennial ryegrass. Seasonal data was not presented for the forages, however, unpublished data from the experiment suggests that the increased DM from plantain was from improved summer and autumn growth. This increase in DM production over summer and autumn was also seen by Nobilly *et al.* (2012) when plantain was sown in a mixture. These results show plantain can produce at least as much DM as perennial ryegrass. Further, plantain can maintain a high quality forage at times of the year when perennial ryegrass-white clover pastures do not perform well (Rawnsley *et al.* 2007). Milk production results presented in Chapter 3 and Chapter 6 this thesis combined forage quality results in Chapter 4 and Chapter 5 and DM production found by Martin *et al.* (2017) show there is considerable potential for plantain to add value to farming systems whilst reducing N leaching risks.

8.6.4 Pasture measurement and allocation

The decision to graze a paddock is based on the pre-grazing mass of the paddock. For perennial ryegrass-white clover pastures this is often assessed using a rising plate meter. The rising plate meter is calibrated to calculate pasture mass according to compressed sward height. Therefore, morphological differences in forages can influence the accuracy of the rising plate meter. Plantain has a lower DM% and more upright growth habit compared with perennial ryegrass-white clover (Stewart *et al.* 2014). In this thesis separate calibration curves were developed for plantain and perennial ryegrass-white clover. These showed differences in slope and intercept of the equations, so giving different predictions of DM for a particular height. Therefore recalibration of rising plate meter for plantain will be required to deliver accurate allocation of feed on farm. This has been carried out by Haultain *et al.* (2014) in the Waikato for first year plantain. Their experiment found that the rising plate meter was a suitable tool for the estimation of biomass in pure plantain swards as it had similar accuracy to calibration equations for a ryegrass-based pasture. The calibration equation by Haultain *et al.* (2014) was used to estimate and allocate plantain forages in Chapter 3 and Chapter 6 of this thesis.

8.7 Experimental design

One of the challenges involved in carrying out a grazing experiment is experimental design. Decisions must be made on how best to create replicated treatments. In Chapter 3, animals were blocked and allocated as groups of 12 to one of three pasture treatments. In this experiment, each sampling day was considered as the replicate. In contrast, Chapter 6 assigned animals from within blocks to graze one of two pasture treatments in three (1.5 ha paddock) replicates of four cows per herd. This meant that each pasture treatment was replicated three times.

According to Bello *et al.* (2016), when the effects of a diet are evaluated on animal production, the paddock or pen in which animals are fed that diet becomes the experimental unit rather than the individual animals within a pen or paddock. Where samples are taken from individual within their treatment group, e.g. milk yield or urine composition data, the animals are considered as pseudoreplicates. However, animals within a paddock are not mutually independent and, consequently, neither are their observations. Therefore, to compare across these treatments, replicates at the group scale (paddock replicates) are required, which was the case for Chapter 6. However, his is not always the most practical approach to experimental design for grazing experiments.

In Chapter 3, replication of pasture treatments was not practical due to the requirement of equipment and labour for a replicated design. Including two replicates into this experiment would have required at least an additional 10 electric fence reels, 3 troughs and extended pasture allocation and sampling time beyond the labour availability at the time. Therefore, the decision to use sampling days as replicates and animals as observational units provided a practical option to carry out and statistically analyse the experiment.

8.8 Opportunities for future work

8.8.1 Modelling

Data from Martin *et al.* (2017) and Chapter 4 showed that DM and ME production on annual and seasonal basis is similar or higher in plantain than perennial ryegrass-white clover pastures, and that nutritional characteristics of the forage were within the ranges suitable for milk production (Waghorn *et al.* 1987). Plantains ability to produce greater quantities of high quality forage leads to the expectation that including plantain within a dairy farm system may indeed increase milk production at the farm scale. Collecting data from a whole farm system experiment would be difficult and expensive. Therefore this hypothesis of plantain increasing milk production at the farm scale could be confirmed by farm systems modelling. Modelling may also be useful to determine how to include plantain into a farm system and how to best deliver it to the animal (as a grazed forage or as a conserved feed).

A feature of the results from this thesis is the ability of plantain to reduce urinary N losses and thus the risk of N leaching. This coupled with results from Woods (2017) which showed a reduction in N leaching under plantain and Italian ryegrass due to increased plant N uptake, reduced N loading and potentially lower nitrification as seen by Massaccesi *et al.* (2015) shows there is a clear benefit in including plantain in a farm system to reduce N leaching into waterways. Regional Councils throughout New Zealand have been developing regulations that place a limit on the amount of N leaching from agricultural land. Currently the nutrient model Overseer is used by regional councils to determine current farm N losses and set farm nutrient loss limits. However, overseer estimations of nutrient losses can vary by 30% and there is currently no option to include plantain as an alternative forage. Therefore, using plantain in a system is not currently recognised by councils as beneficial tool to reduce N losses. In order for plantain to be included in the Overseer model further data is required around N leaching losses under plantain. This would include data presented in this thesis quantifying urinary N concentration from plantain and would also require information around urination behaviour of cows grazing plantain. Urination behaviour from cows grazing plantain could be used to model how many urinations fall on a per hectare scale, the likelihood of urine patch overlap and therefore the overall N deposition per hectare (Selbie *et al.* 2015). This would allow for the calculation of the overall risk of N leaching at a paddock and farm scale when plantain is included in an animal’s diet.

8.8.2 Plantain secondary metabolite concentration

The confounding results in this study on secondary plant metabolite concentration in plantain herbage represents an area for future work. In Chapter 3 the concentration of metabolites appeared to be greater in autumn than spring. However, in Chapter 7 the opposite appeared to be true. Further, the mechanisms which drive plant secondary metabolite concentration in plantain herbage were unclear. Previous studies carried out in Japan suggest temperature and light intensity can drive the production of secondary metabolites (Tamura 2001). However, this was not measured in the current study. This represents an area where future work is required to determine what drives secondary plant compound concentrations in plantain herbage and thresholds for metabolite production.

8.8.3 Milk composition

Milk composition is important to consider as it has implications on product quality (Auldist *et al.* 1998; Lock *et al.* 2004). Milk composition can be influence by nutrition (Lock *et al.* 2004; Cersosimo *et al.* 2016). Therefore, offering plantain as an alternative forage may influence the milk composition. With the exception of a small decrease in milk fat in late lactation, Chapter 3 found that plantain has little influence on milk composition compared with perennial ryegrass-white clover. However, this experiment did not measure milk fat composition. Milk fat consists mainly of triglycerides (approximately 98%), while other milk lipids are diacylglycerol (about 2% of the lipid fraction), cholesterol (less than 0.5%), phospholipids (about 1%) and free fatty acids (FFA) (about 0.1) (Jensen *et al.* 1995). The milk fatty acid profile has consequences on product manufacturing. Diet is known to influence the fatty acid profile of milk. Recently due to the effect of palm kernel expeller (PKE) on the milk fatty acid profile, dairy co-op, Fonterra, has rolled out a Fat Evaluation Index (FEI) with a demerit based grading system being rolled out in June 2018 (Christian 2017). Under this demerit system an FEI of over 8 exceeds Fonterra's threshold limit and will result in a C grade, above 9.5 would result in a D grade. The implications of these grades has not yet been announced. The milk fat composition from cows grazing plantain has not previously been determined. However, research using diverse pastures which contained plantain found that the proportion (g/100g of milk fat fatty acids) of linoleic and linolenic acid increased while the proportion of cis-9, trans-11 C18:2 (CLA) decreased in milk from cows grazing the diverse pastures compared to cows fed perennial ryegrass-white clover pastures (Ström 2012). The author concluded that the changes were likely associated with a shift in the rumen microbial population or rumen metabolic routes caused by several secondary metabolites present in some plants. Given plantain's secondary plant metabolite composition, it is likely pure plantain swards could result in a change in milk fatty acid profiles. Future work should measure the milk fat composition from cows grazing plantain in order to determine the FEI of milk from cows grazing plantain and therefore whether feeding plantain has the potential to result in demerits under a milk companies grading system.

8.8.4 Cultivar differences

This thesis showed the potential of plantain cv Tonic to reduce urinary N losses whilst maintaining milk production. Cultivars other than Tonic were not included in this study. There may be within cultivar differences. There is currently little published data which compares animals production and urinary N from different cultivars of plantain. A recent unpublished study has compared urine output of sheep grazing twelve different commercial cultivars of plantain (Judson, G., Unpublished). Water intake was balanced for all animals. The experiment found differences in urine output amoung cultivars. This suggests the directic effect of plantain can be dependent on the cultivar used. Further research to determine the most suitable cultivar for reducing urinary N losses while maintaing DM and milk prodution would be useful to determine which cultivar should be sown on farms.

8.9 Conclusions

The research presented in this thesis has provided a comprehensive assessment on the use of plantain in comparison to perennial ryegrass-white clover pastures for milk production and urinary N output and the diurnal changes in chemical composition of forages.

Specific conclusions were:

- Milk production could be maintained or improved (through increased DM intake) when plantain was included at 100 or 50% of the diet, with large reductions in urinary N and urine N output. This represents a significant potential for plantain to reduce the risk of N leaching from dairy systems without impeding production.

- Diurnal changes in plantain chemical composition were greater for perennial ryegrass than for plantain. This suggest diurnal allocation, where plantain was allocated in the afternoon would not provide any benefits.

- Allocation of plantain in the morning as 50% of the diet and perennial ryegrass-white clover in the afternoon as 50% of the diet did not improve NUE or milk production from dairy cows in late lactation.

- Plantain may not contain sufficient levels of the diuretic catalpol to have an effect on the grazing animal. However acteoside and aucubin were present in plantain herbage. These secondary plant metabolites may be useful for reducing NH_4 production in the rumen and NO_{3-} accumulation in the soil however further research is required.

References

Allen MS 2014. Drives and limits to feed intake in ruminants. *Animal Production Science 54*(10), 1513-1524. doi: https://doi.org/10.1071/AN14478

Andary C, Wylde R, Laffite C, Privat G, Winternitz F 1982. Structures of verbascoside and orobanchoside, caffeic acid sugar esters from Orobanche rapum-genistae. *Phytochemistry 21*(5), 1123-1127. doi: https://doi.org/10.1016/S0031-9422(00)82429-2

Auldist MJ, Walsh BJ, Thomson NA 1998. Seasonal and lactational influences on bovine milk composition in New Zealand. *Journal of Dairy Research 65*(3), 401-411. doi: undefined

Bauman DE, Currie WB 1980. Partitioning of Nutrients During Pregnancy and Lactation: A Review of Mechanisms Involving Homeostasis and Homeorhesis. *Journal of Dairy Science 63*(9), 1514-1529.

Belesky DP, Ruckle JM, Clapham WM 2004. Dry-Matter Production, Allocation and Nutritive Value of Forage Chicory Cultivars as a Function of Nitrogen. *Journal of Agronomy and Crop Science 190*(2), 100-110. doi: 10.1046/j.1439-037X.2003.00081.x

Bello NM, Kramer M, Tempelman RJet al. 2016. Short communication: On recognizing the proper experimental unit in animal studies in the dairy sciences. *Journal of Dairy Science 99*(11), 8871-8879. doi: https://doi.org/10.3168/jds.2016-11516

Betteridge K, Andrewes W, Sedcole J 1986. Intake and excretion of nitrogen, potassium and phosporus by grazing steers. *Journal of Agricultural Science 106*(2), 393-404. doi: 10.1017/S0021859600064005

Betteridge K, Costall DA, Li FY, Luo D, Ganesh S 2013. Why we need to know what and where cows are urinating – a urine sensor to improve nitrogen models. *Proceedings of the New Zealand Grassland Association 75*, 119-124.

Beukes PC, Gregorini P, Romera AJet al. 2014. The potential of diverse pastures to reduce nitrogen leaching on New Zealand dairy farms. *Animal Production Science 54*(12), 1971-1979. doi: https://doi.org/10.1071/AN14563

Bourgaud F, Gravot A, Milesi S, Gontier E 2001. Production of plant secondary metabolites: a historical perspective. *Plant Science 161*(5), 839-851. doi: http://dx.doi.org/10.1016/S0168-9452(01)00490-3

Bowers MD, Collinge SK, Gamble SE, Schmitt J 1992. Effects of genotype, habitat and seasonal variation on iridoid glycoside content of *Plantago lanceolata* and the implications for insect herbivores. *Oecologia 91*, 201-207.

Box LA, Edwards GR, Bryant RH 2016. Milk production and urinary nitrogen excretion of dairy cows grazing perennial ryegrass-white clover and pure plantain pastures. *Proceedings of the New Zealand Society of Animal Production 76*, 18-21.

Bryant JR, Hoogendoorn CJ, Snow VO 2007. Simulation of mitigation strategies to reduce nitrogen leaching from grazed pasture. *Proceedings of the New Zealand Grassland Association 69*, 145-151.

Bryant RH, Gregorini P, Edwards GR 2012. Effects of N fertilisation, leaf appearance and time of day on N fractionation and chemical composition of Lolium perenne cultivars in spring. *Animal Feed Science and Technology 173*, 210-219.

Bryant RH, Dalley DE, Gibbs J, Edwards GR 2013. Effect of grazing management on herbage protein concentration, milk production and nitrogen excretion of dairy cows in mid-lactation. *Grass and Forage Science 69*(4), 64-654.

Bryant RH, Dalley DE, Gibbs J, Edwards GR 2014. Effect of grazing management on herbage protein concentration, milk production and nitrogen excretion of dairy cows in mid-lactation. *Grass and Forage Science 69*, 644-654.

Bryant RH, Miller LA, Greenwood SL, Edwards GR 2017. Milk yield and nitrogen excretion of dairy cows grazing binary and multispecies pastures. *Grass and Forage Science 00*, 1-12. doi: 10.1111/gfs.12274

Burke JL, Waghorn GC, Chaves AV 2002. Improving animal performance using forage-based diets. *Proceedings of the New Zealand Society of Animal Production 62*, 267-272.

Burke JL, Waghorn GC, Brookes IM, Attwood GT, Kolver ES 2000. Formulating total mixed rations from forages - defining the digestion kinetics of contrasting species. *Proceedings of the New Zealand Society of Animal Production 60*, 9-14.

Cameron KC, Di HJ, Moir J 2013. Nitrogen losses from the soil/plant system: a review. *Annals of Applied Biology 162*, 145-173.

Castillo AR, Kebreab E, Beever DE, France J 2000. A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. *Journal of Animal and Feed Sciences 9*, 1-32.

Cersosimo LM, Tacoma R, Greenwood S, Juntwait K, Brito AF, Kraft J 2016. Alternative forage crops modify the composition and content of bovine milk fatty acids. *Journal of Animal Science 94*(supplement5), 687-687. doi: 10.2527/jam2016-1417

Charlton JFL, Stewart AV 1999. Pasture species and cultivars used in New Zealand – a list. *Proceedings of the New Zealand Grassland Association 61*, 147-166.

Chaves AV, Burke JL, Waghorn GC, Brookes IM 2006. Digestion kinetics of leaf, stem and inflorescence from five species of mature grasses. *Journal of the Science of Food and Agriculture 86*(5), 816-825. doi: 10.1002/jsfa.2420

Chen CH, Lin YS, Chien MY, Hou WC, Hu ML 2012. Antioxidant and antihypertensive activities of acteoside and its analogs. *Botanical Studies 53*(4), 421-429.

Chen XB 1989. Excretion of purine derivatives by sheep and cattle and its use for the estimation of absorbed microbial protein. Unpublished thesis, University of Aberdeen, Aberdeen, Scotland.

Cheng L, Judson HG, Bryant RHet al. 2017. The effects of feeding cut plantain and perennial ryegrass-white clover pasture on dairy heifer feed and water intake, apparent nutrient digestibility and nitrogen excretion in urine. *Animal Feed Science and Technology 229*(Supplement C), 43-46. doi: https://doi.org/10.1016/j.anifeedsci.2017.04.023

Chizzotti ML, Valadares Filho SC, Valadares RFD, Chizzotti FHM, Tedeschi LO 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. *Livestock Science 113*, 218-225.

Christian G 2017 The big fat issue: Demerits ahead. Dairy Exporter: 35-42.

Cosgrove GP, Koolaard J, Luo D, Burke JL, Pacheco D 2009. The composition of high sugar ryegrasses. *Proceedings of the New Zealand Grassland Association 71*, 187-193.

Cosgrove GP, Taylor PS, Lowe KA, Foote AG, Jonker A 2014. Milk urea estimates of nitrogen excretion by dairy cows grazing forage species with contrasting chemical and morphological characteristics. *Proceedings of the 5th Australasian Dairy Science Symposium*, 249-251.

Cosgrove GP, Pacheco D, Burke JL, Death AF, Hickey MJ, Lane GA 2007a. Effects of water soluble carbohydrate in forage on aspects of nitrogen partitioning in cows. In: Chapman DF, Clark DA, Macmillan KL, Nation DP ed. *Meeting the challenges for pasture-based dairying. Proceedings of the 3rd Australian Dairy Science Symposium*. Pp. 350-358.

Cosgrove GP, Burke JL, Death AF, Hickey MJ, Pacheco D, Lane GA 2007b. Ryegrasses with increased water soluble carbohydrate: evaluating the potential for grazing dairy cows in New Zealand. *Proceedings of the New Zealand Grassland Association 69*, 179-185.

Cosgrove GP, Burke JL, Death AFet al. 2006. Milk production by dairy cows grazing mixed swards of adjacent monocultures of grass and white clover. *Proceedings of the New Zealand Society of Animal Production 66*, 42-49.

Cranston LM, Kenyon PR, Morris ST, Lopez-Villalobos N, Kemp PD 2016. Morphological and Physiological Responses of Plantain (Plantago lanceolata) and Chicory (Cichorium intybus) to Water Stress and Defoliation Frequency. *Journal of Agronomy and Crop Science 202*(1), 13-24. doi: 10.1111/jac.12129

CSIRO ed. 2007. Nutrient requirements of domesticated ruminants. Collingwood, Australia.

Czauderna M, Kowalczyk J 2000. Quantification of allantoin, uric acid, xanthine and hypoxanthine in ovine urine by high-performance liquid chromatography and photodiode array detection. *Journal Chromatography B Biomedical Sciences and Applications 744*(1), 129-38.

DairyNZ 2017. Facts and figures for New Zealand dairy farmers, 2nd Edition. Hamilton, DairyNZ. 163 p.

Daly MJ, Hunter RM, Green GN, Hunt LA 1996. A comparison of multi-species pasture with ryegrass-white clover pasture under dryland conditions. *Proceedings of the New Zealand Grassland Association 58*, 53-58.

Deaker JM, Young MJ, Fraser TJ, Rowarth JS 1994. Carcuss, liver and kidney characteristics of lambs grazing plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*), white clover or perennial ryegrass. *Proceedings of New Zealand Society of Animal Production 120*, 51-61.

DeGaris PJ, Lean IJ 2008. Milk fever in dairy cows: A review of pathophysiology and control principles. *The Veterinary Journal 176*(1), 58-69. doi: https://doi.org/10.1016/j.tvjl.2007.12.029

Dewhurst RJ, Davies DR, Merry RJ 2000. Microbial protein supply from the rumen. *Animal Feed Science and Technology 85*(1), 1-21. doi: http://dx.doi.org/10.1016/S0377-8401(00)00139-5

Di HJ, Cameron KC 2001. Effects of the nitrification inhibitor dicyandiamide on potassium, magnesium and calcium leaching in grazed grassland. *Soil Use and Management*, 2-7.

Di HJ, Cameron KC 2002a. Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. *Nutrient Cycling in Agroecosystems 46*(3), 237-256. doi: 10.1023/A:1021471531188

Di HJ, Cameron KC 2002b. The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. *Soil Use and Management 18*(395-403).

Di HJ, Cameron KC 2007. Nitrate leaching losses and pasture yields as affected by different rates of animal urine nitrogen returns and application of a nitrification inhibitor - a lysimeter study. *Nutrient Cycling and Agroecosystems 79*, 281-290.

Dietz M, Machill S, Hoffmann HC, Schmidtke K 2013. Inhibitory effects of Plantago lanceolata L. on soil N mineralization. *Plant and Soil 368*(1), 445-458. doi: 10.1007/s11104-012-1524-9

Dijkstra J, Oenema O, van Groenigen JW, Spek JW, van Vuuren AM, Bannink A 2013. Diet effects on urine composition of cattle and N2O emissions. *Animal 7 Suppl 2*, 292-302. doi: 10.1017/s1751731113000578

Edwards GR, Lucas RJ, Johnson MR 1993. Grazing preference for pasture species by sheep is affected by endophyte and nitrogen fertility. *Proceedings of the New Zealand Grassland Association 55*, 137-141.

Edwards GR, Parsons AJ, Rasmussen S 2007. High sugar ryegrasses for dairy systems. *Meeting the Challenges for Pasture-Based Dairying - Proceedings of the Australasian Dairy Science Symposium*. Pp. 307-334.

Edwards GR, Parsons AJ, Bryant RH 2008. Manipulating dietary preference to improve animal performance. *Australian Journal of Experimental Agriculture 48*(7), 773-779. doi: http://dx.doi.org/10.1071/EA08006

Edwards GR, Bryant RH, Smith Net al. 2015. Milk production and urination behaviour of dairy cows grazing diverse and simple pastures. *Proceedings of the New Zealand Society of Animal Production 75*, 79-83.

Elgersma A, Hassink J 1997. Effects of white clover (*Trifolium repens* L.) on plant and soil nitrogen and soil organic matter in mixtures with perennial ryegrass (*Lolium perenne* L.). *Plant and Soil 197*(2), 177-186. doi: 10.1023/a:1004237527970

Ellis JL, Dijkstra J, Bannink Aet al. 2011. The effect of high-sugar grass on predicted nitrogen excretion and milk yield simulated using a dynamic model. *Journal of Dairy Science 94*(6), 3105-3118. doi: 10.3168/jds.2010-4059

Fajer ED, Bowers MD, Bazzaz FA 1992. The Effect of Nutrients and Enriched CO2 Environments on Production of Carbon-Based Allelochemicals in Plantago: A Test of the Carbon/Nutrient Balance Hypothesis. *The American Naturalist 140*(4), 707-723. doi: 10.1086/285436

Farrell LJ, Bryant RH, Hague H, Edwards GR 2016. Urine volume of non-lactating dairy cows in late gestation fed forage based diets in winter. *Proceedings of the New Zealand Society of Animal Production 76*, 22-25.

Fraser TJ, Rowarth JS 1996a. Legumes, herbs or grass for lamb performance? *Proceedings of the New Zealand Grassland Association 49*, 49-52.

Fraser TJ, Scott SM, Rowarth JS 1996b. Pasture species effects on carcuss and meat quality. *Proceedings of the New Zealand Grassland Association 58*, 63-66.

Fulkerson WJ, Donaghy DJ 2001. Plant-soluble carbohydrate reserves and senescence - key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental Agriculture 41*(2), 261-275. doi: http://dx.doi.org/10.1071/EA00062

Gardiner CA, Clough TJ, Cameron KC, Di HJ, Edwards GR, de Klein CAM 2016. Potential for forage diet manipulation in New Zealand pasture ecosystems to mitigate ruminant urine derived N2O emissions: a review. *New Zealand Journal of Agricultural Research 59*(3), 301-317. doi: 10.1080/00288233.2016.1190386

Gawn TL, Harrington KC, Matthew C 2012. Weed control in establishing mixed swards of clover, plantain and chicory. *New Zealand Plant Protection Society 65*, 59-63.

George SK, Dipu MT, Mehra UR, Singh P, Verma AK, Ramgaokar JS 2006. Improved HPLC method for the simultaneous determination of allantoin, uric acid and creatinine in cattle urine. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences 832*(1), 134-137. doi: 10.1016/j.jchromb.2005.10.051

Golding KP, Kemp PD, Kenyon PR, Morris ST 2008. High weaned lamb live weight gains on herbs. *Agronomy New Zealand 38*, 33-39.

Gregorini P, Beukes P, Bryant RH, Romera A 2010. A brief overview and simulation of the effects of some feeding strategies on nitrogen excretion and enteric methane emission from grazing dairy cows. In: Edwards GR, Bryant RH ed. *Meeting the challenges for pasture-based dairying. Proceedings of the 4th Australasian Dairy Science Symposium.* Pp. 29-43.

Groenigen JW, Velthof GL, Oenema O, Groenigen KJ, Kessel C 2010. Towards an agronomic assessment of N2O emissions: a case study for arable crops. *European Journal of Soil Science 61*, 909-913.

Hammond J 1982. Plantago As a Host of Economically Important Viruses. In: Lauffer MA, Bang FB, Maramorosch K, Smith KM ed. Advances in Virus Research, Academic Press. Pp. 103-140.

Haultain J, Wigley K, Lee JM 2014. Rising plate meters and a capacitance probe estimate the biomass of chicory and plantain monocultures with similar accuracy as for ryegrass-based pasture. *Proceedings of the New Zealand Grassland Association 76*, 67-74.

Haynes RJ, Williams PH 1993. Nutrient Cycling and Soil Fertility in the Grazed Pasture Ecosystem. *Advances in Agronomy 49*, 119-199. doi: http://dx.doi.org/10.1016/S0065-2113(08)60794-4

Higgs RJ, Chase LE, van Amburgh ME 2012. Development and evaluation of equation sin the Cornell Net Carbohydrate and Protein System to predict nitrogen excretion in lactating dairy cows. *Journal of Dairy Science 95*, 2004-2014.

Hoekstra NJ, Schulte RPO, Struik PC, Lantinga EA 2007. Pathways to improving the N efficiency of grazing bovines. *European journal of agronomy 26*, 363-374.

Holmes CW, Roche JR 2007. Pastures and supplements in dairy production systems. In: Rattray PV, Brookes IM, Nicol AM ed. Pasture and Supplements for grazing animals, New Zealand Society of Animal Production Occasional Publication. Pp. 221-242.

Hoskin SO, Wilson PR, Ondris M, Bunod AH 2006. The Feeding Value of Forage Herbs: Studies with Red Deer. *Proceedings of the New Zealand Grasslands Association 68*, 199-204.

Hristov A, Ropp J, Grandeen Ket al. 2005. Effect of carbohydrate source on ammonia utilization in lactating dairy cows. *Journal of Animal Science 83*, 408–421.

Hutton PG, Kenyon PR, Bedi MKet al. 2011. A herb and legume sward mix increased ewe milk production and ewe and lamb live weight gain to weaning compared to a ryegrass dominant sward. *Animal Feed Science and Technology 164*(1), 1-7. doi: http://dx.doi.org/10.1016/j.anifeedsci.2010.11.014

Jarvis SC, Scholefield D, Pain B 1995. Nitrogen cycling in grazing systems. In: Bacon PE ed. Nitrogen Fertilization in the Environment. New York, Marcel Dekker Inc. Pp. 381-419.

Jensen RG, Newburg DS 1995. Bovine milk lipids. Jensen RG ed. London, UK, Academic Press.

John A, Ulyatt MJ 1987. Importance of dry matter content to voluntary intake of fresh grass forages. *Proceedings of the New Zealand Society of Animal Production 47*, 13-16.

Jones TA, Carlson IT, Buxton DR 1988. Reed Canarygrass Binary Mixtures with Alfalfa and Birdsfoot Trefoil in Comparison to Monocultures. *Agronomy Journal 80*(1), 49-55. doi: 10.2134/agronj1988.00021962008000010011x

Jonker JS, Kohn RA, Erdman RA 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. *Journal of Dairy Science 81*, 2681-2692.

Judson GJ 2008. Preliminary evaluation of plantain (*Plantago lanceolata*) cultivar Tonic as a feed for ewe lactation. In: Boschma S, Serafin L, Ayres J ed. *Pastures at the cutting edge : proceedings of the 23rd annual conference of the Grassland Society of NSW*. Pp. 130-131.

Judson GJ, McFarlane JD 1998. Mineral disorders in grazing livestock and the usefulness of soil and plant analysis in the assessment of these disorders. *Australian Journal of Experimental Agriculture 38*(7), 707-723. doi: https://doi.org/10.1071/EA97145

Judson HG, Edwards GR 2016. Urinary nitrogen concentration from dairy heifers grazing kale supplemented with either plantain or perennial ryegrass baleage in winter. *Journal of New Zealand Grasslands 78*, 99-102.

Judson HG, McAnulty R, Sedcole R 2009. Evaluation of ‘Ceres Tonic’ plantain (*Plantago lancelota*) as a lactation feed for twin bearing ewes. *Proceedings of the New Zealand Grassland Association 71*, 201-205.

Kato Y 1946. Mechanism of uric acid excretion stimulation by aucubin. *Folia Phamacol 42*, 37-40.

Kebreab E, France J, Beever DE, Castillo AR 2001. Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. *Nutrient Cycling in Agroecosystems 60*(1), 275-285. doi: 10.1023/a:1012668109662

Keir BL, Mayes RW, Orskov ER 2001. The potential of urinary metabolites of plant compounds as indicators of botanical composition of the diet of goats. *Proceedings of the British Society of Animal Science, Annual Meeting, 9-11 April 2001*.

Kemp PD, Matthews PNP, Lucas RJb 1999. Pasture species and cultivars. In: White JGH, Hodgson J ed. Zealand Pasture and Crop Science. Auckland, New Zealand, Oxford University. Pp. 83-99.

Kemp PD, Kenyon PR, Morris ST 2010. The use of legume and herb forage species to create high performance pastures for sheep and cattle grazing systems. *Revista Brasileira de Zootecnia 39*, 169-174.

Kenyon PR, Kemp PD, Stafford KJ, West DM, Morris ST 2010. Can a herb and white clover mix improve the performance of multiple-bearing ewes and their lambs to weaning? *Animal Production Science 50*, 513-521.

Ledgard SF, Welten B, Betteridge K 2015. Salt as a mitigation option for decreasing nitorgen leaching losses from grazed pastures. *Journal of the Science of Food and Agriculture 95*(15), 3033–3040. doi: 10.1002/jsfa.7179

Ledgard SF, Welten BG, Menneer JC, Betteridge K, Crush JR, Barton MD 2007. New nitrogen mitigation technologies for evaluation in the Lake Taupo catchment. *Proceedings of the New Zealand Grassland Association 69*, 117-121.

Levy EB 1970. Grasslands of New Zealand. 3 ed. Wellington, Shearer, A.R., Government Printer

Lock AL, Bauman DE 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids 39*(12), 1197-206.

Malcolm BJ, Cameron KC, Di HJ, Edwards GR, Moir JL 2014. The effect of four different pasture species compositions on nitrate leaching losses under high N loading. *Soil Use and Management 30*(1), 58-68.

Martin KM, Edwards GR, Bryant RHet al. 2017. Herbage dry-matter yield and nitrogen concentration of grass, legume and herb species grown at different nitrogen-fertiliser rates under irrigation. *Animal Production Science*, -. doi: https://doi.org/10.1071/AN16455

Massaccesi L, Bardgett RD, Agnelli A, Ostle N, Wilby A, Orwin KH 2015. Impact of plant species evenness, dominant species identity and spatial arrangement on the structure and functioning of soil microbial communities in a model grassland. *Oecologia 177*(3), 747-59. doi: 10.1007/s00442-014-3135-z

McFarlane AW 1990. Field experience with new pasture cultivars in Canterbury. *Proceedings of the New Zealand Grassland Association 52*, 139-143.

McLaren RG, Cameron KC 1996. Soil science: sustainable production and environmental protection. Wellington, Oxford University Press.

Miller LA, Theodorou MK, MacRae JCet al. 1999. Milk production and N partitioning responses in dairy cows offered perennial ryegrass selected for high water soluble carbohydrate concentrations. *IX International Symposium on Ruminant Physiology*. Pp. 281-289.

Miller LA, Theodorou MK, MacRae JCet al. 2000. Efficiency of nitrogen use by dairy cows offered perennial ryegrass with high water soluble carbohydrate concentrations. *Proceedings of the 6th Research Conference of the British Grassland Society*. Pp. 37-38.

Miller LA, Moorby JM, Davies DRet al. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne L*): milk production from late-lactation dairy cows. *Grass and Forage Science 56*(4), 383-394.

Minneé EMK, Clark CEF, Clark DA 2013. Herbage production from five grazable forages. *Proceedings of the New Zealand Grassland Association 75*, 245-250.

Minneé EMK, Waghorn GC, Lee JM, Clark CEF 2017. Including chicory or plantain in a perennial ryegrass/white clover-based diet of dairy cattle in late lactation: Feed intake, milk production and rumen digestion. *Animal Feed Science and Technology 227*, 52-61. doi: https://doi.org/10.1016/j.anifeedsci.2017.03.008

Misselbrooka T, Fleminga H, Campa Vet al. 2016. Automated monitoring of urination events from grazing cattle. *Agriculture, Ecosystems and Environment 230*, 191-198.

Moir JL, Edwards GR, Berry LN 2013. Nitrogen uptake and leaching loss of thirteen temperate grass species under high N loading. *Grass and Forage Science 68*, 313–325.

Moorby JM, Evans RT, Scollan ND, MacRae JC 2006. Increased concentration of water-soluble carbohydrate in perennial ryegrass (Lolium perenne L.). Evaluation in dairy cows in early lactation. *Grass and Forage Science 61*, 52-59.

Moorhead AJE, Piggot GJ 2009. The performance of pasture mixes containing 'Ceres Tonic' plantain (Plantago lanceolata) in Northland. *Proceedings of the New Zealand Grassland Association 71*, 195-199.

Moorhead AJE, Judson GJ, Stewart AV 2002. Liveweight gain of lambs grazing 'Ceres Tonic' plantain (Plantago lanceolata) or perennial ryegrass (Lolium perenne). *New Zealand Society of Animal Production 62*, 171-173.

Mueller-Harvey I 2006. Unravelling the conundrum of tannins in animal nutrition and health. *Journal of the Science of Food and Agriculture 86*(13), 2010-2037. doi: 10.1002/jsfa.2577

Navarrete S, Kemp PD, Pain SJ, Back PJ 2016. Bioactive compounds, aucubin and acteoside, in plantain (Plantago lanceolata L.) and their effect on in vitro rumen fermentation. *Animal Feed Science and Technology 222*, 158-167.

Neal JS, Fulkerson WJ, Lawrie R, Barchia IM 2009. Difference in yield and persistence among perennial forages used by the dairy industry under optimum and deficit irrigation. *Crop and Pasture Science 60*(11), 1071-1087. doi: https://doi.org/10.1071/CP09059

Nishibi S, Mural M 1995. Bioactive components of plantago herb. *Foods and food ingredients journal 166*, 43-49.

Nkomboni D 2017. Effect of plantain (*Plantago lanceolata* L.) proportion in the diet on nitrogen use, milk production and behaviour of lactating dairy cows. Unpublished thesis, Lincoln University. 77 p.

Nobilly F, Bryant RH, McKenzie BA, Edwards GR 2012. Productivity of rotationally grazed simple and diverse pasture mixtures under irrigation in Canterbury. *Proceedings of the New Zealand Grassland Association 75*, 165-171.

Nuthall R, Rutter SM, Rook AJ 2000. Milk production by dairy cows grazing mixed swards of adjacent monocultures of grass and white clover. *Proceedings of the 6th Reasearch Conference*. Pp. 37-38.

O'Connell CA, Judson HG, Barrell GK 2016. Sustained diuretic effect of plantain when ingested by sheep. *Proceedings of the New Zealand Society of Animal Production 76*, 14-17.

Ørskov ER, McDonald I 2009. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *The Journal of Agricultural Science 92*(2), 499-503. doi: 10.1017/S0021859600063048

Ørskov ER, Hovell FDD, Mould FTAP 1980. The use of the nylon bag technique for the evaluation of feedstuffs. *Tropical Animal Production 5*(3), 195-213.

Pacheco D, Waghorn GC 2008. Dietary nitrogen definitions, digestion, excretion and consequences of excess for grazing ruminants. *Proceedings of the New Zealand Grassland Association 70*, 107-116.

Parsons AJ, Rasmussen S, Xue H, Newman JA, Anderson CB, Cosgrove GP 2004. Some 'high sugar grasses' don't like it hot. *Proceeding of the New Zealand Grassland Association 66*, 265-271.

Pembleton KG, Hills JL, Freeman MJ, McLaren DK, French M, Rawnsley RP 2016. More milk from forage: Milk production, blood metabolites, and forage intake of dairy cows grazing pasture mixtures and spatially adjacent monocultures. *Journal of Dairy Science 99*(5), 3512-3528. doi: http://dx.doi.org/10.3168/jds.2015-10542

Petersen SO, Sommer SG, Aaes O, Søegaard K 1998. Ammonia losses from urine and dung of grazing cattle. *Atmospheric Environment 32*(3), 295-300. doi: http://dx.doi.org/10.1016/S1352-2310(97)00043-5

Pirhofer-Walzl K, Søegaard K, Høgh-Jensen Het al. 2011. Forage herbs improve mineral composition of grassland herbage. *Grass and Forage Science 66*(3), 415-423. doi: 10.1111/j.1365-2494.2011.00799.x

Powell AM, Kemp PD, Jaya ID, Osbourne MA 2007. Establishment, growth and development of plantain and chicory under grazing. *Proceedings of the New Zealand Grassland Association 69*, 41-45.

Rasmussen S, Parsons AJ, Xue H, Newman JA 2009. High sugar grasses - harnessing the benefits of new cultivars through growth management. *Proceedings of the New Zealand Grassland Association 71*, 161-175.

Ravera B, Bryant RH, Cameron KC, Di HJ, Edwards GR, Smith N 2015. Use of a urine meter to detect variation in urination behaviour of dairy cows on winter crops. *Proceedings of the New Zealand Society of Animal Production 75*, 84-88.

Rawnsley RP, Donaghy DJ, Stevens DR 2007. What is limiting production and consumption of perennial ryegrass in temperate dairy regions of Australia and New Zealand. In: Chapman DF, Clark DA, Macmillan KL, Nation DP ed. *Proceedings of the 3rd Australasian dairy science symposium 2007: meeting the challenges for pasture-based dairying*. Pp. 256-274.

Rooke JA, Lee NH, Armstrong DG 1987. The effects of intraruminal infusions of urea, casein, glucose syrup and a mixture of casein and glucose syrup on nitrogen digestion in the rumen of cattle receiving grass-silage diets. *British Journal of Nutrition 57*(1), 89-98.

Rugoho I, Gourley CJP, Hannah MC 2017. Nutritive characteristics, mineral concentrations and dietary cation–anion difference of feeds used within grazing-based dairy farms in Australia. *Animal Production Science 57*(5), 858-876. doi: https://doi.org/10.1071/AN15761

Rumball W, Keogh RG, Lane GE, Miller JE, Claydon RB 1997. "Grasslands Lancelot" plantain (*Plantago lancelata* L.). *New Zealand Journal of Agricultural Research 40*(3), 373-377.

Russell JB, O'Connor JD, Fox DG, Van Soest PJ, Sniffen CJ 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *Journal of animal science 70*(11), 3551-3561. doi: 10.2527/1992.70113551x

Rutter SM, Young KL, Cook JE, Champion RA 2003. Strip-grazing separate white clover and ryegrass monocultures increases daily intake and milk yield in dairy cows. *Tropical and subtropical agroecosystems 3*, 461-465.

Sanderson MA, Labreveux M, Hall MH, Elwinger GF 2003. Nutritive Value of Chicory and English Plantain Forage. *Crop Science 43*(5), 1797-1804. doi: 10.2135/cropsci2003.1797

Sanderson MA, Soder KJ, L.D. M, Klement KD, Skinner RH, Goslee SC 2005. Forage Mixture Productivity and Botanical Composition in Pastures Grazed by Dairy Cattle. *Agronomy Journal 97*, 1465–1471. doi: 10.2134/agronj2005.0032

Scales GH, Knight TL, Saville DJ 1995. Effect of herbage species and feeding level on internal parasites and production performance of grazing lambs. *New Zealand Journal of Agricultural Research 38*(2), 237-247. doi: 10.1080/00288233.1995.9513124

Selbie DR, Buckthought LE, Shepherd MA 2015. The challenge of the urine patch for managing nitrogen in grazed pasture systems. *Advances in Agronomy 129*, 229-292.

Smith KF, Simpson RJ, Oram RNet al. 1998. Seasonal variation in the herbage yield and nutritive value of perennial ryegrass (Lolium perenne L.) cultivars with high or normal herbage water-soluble carbohydrate concentrations grown in three contrasting Australian dairy environments. *Australian journal of experimental agriculture 38,* 821-830.

Stewart AV 1996. Plantain (*Plantago lanceolata*) - a potential pasture species. *Proceedings of the New Zealand Grassland Association 58*, 77-86.

Stewart AV, Kerr G, Lissaman W, Rowarth J 2014. Pasture and forage plants for New Zealand. 4 ed. Dunedin, New Zealand Grasslands Association. 139 p.

Ström G 2012. Effect of botanically diverse pastures on the milk fatty acid profiles in New Zealand dairy cows. Unpublished Masters thesis, Swedish University of Agricultural Sciences. 43 p.

Sun XZ, Waghorn GC 2012. Improving in sacco incubation technique to evaluate fresh forage for selecting fast-degrading perennial ryegrass (Lolium perenne L.). *Grass and Forage Science 67*(3), 437-445. doi: 10.1111/j.1365-2494.2012.00860.x

Sun XZ, Waghorn GC, Clark H 2010. Composition and in sacco degradation characteristics of winter growth of Lolium perenne, Lolium multiflorum and Lolium perenne × multiflorum ryegrasses. *Proceedings of the New Zealand Society of Animal Production 70*, 206-212.

Sutton JD, Oldham JD, Hart IC 1980. Products of digestion, hormones and energy utilization in milking cows given concentrates containing varying proportions of barley or maize. *Publication, European Association for Animal Production*(No. 26), 303-306.

Tamminga S 1992. Nutrition management of dairy cows as a contribution of pollution control. *Journal of Dairy Science 75*, 345-357.

Tamminga S 1996. A review on environmental impacts of nutritional strategies in ruminants. *Journal of Animal Science 74*, 3112-3124.

Tamura Y 2001. Effects of temperature, shade, and nitrogen application on the growth and accumulation of bioactive compounds in cultivars of *Plantago lanceolata* L. *Japanese Journal of Crop Science 70*(4), 548-553. doi: 10.1626/jcs.70.548

Tamura Y, Nishibe S 2002. Changes in the Concentration of Bioactive Compounds in Plantain Leaves. *Journal of agricultural and food chemistry 50*, 2514-2518.

Tas BM, Taweel HZ, Smit HJ, Elgersma A, Dijkstra J, Tamminga S 2006a. Rumen degradation characteristics of perennial ryegrass cultivars during the growing season. *Animal Feed Science and Technology 131*(1), 103-120. doi: http://dx.doi.org/10.1016/j.anifeedsci.2006.02.002

Tas BM, Taweel HZ, Smit HJ, Elgersma A, Dijkstra J, Tamminga S 2006b. Utilisation of N in perennial ryegrass cultivars by stall-fed lactating dairy cows. *Livestock Science 100*, 159-168. doi: 10.1016/j.livsci.2005.08.013

Tharmaraj J, Chapman DF, Nie ZN, Lane AP 2008. Herbage accumulation, botanical composition, and nutritive value of five pasture types for dairy production in southern Australia. *Australian Journal of Agricultural Research*(59), 127-138.

Tharmaraj J, Chapman DF, Hill J, Jacobs JL, Cullen BR 2014. Increasing home-grown forage consumption and profit in non-irrigated dairy systems. 2. Forage harvested. *Animal Production Science 54*(3), 234-246. doi: https://doi.org/10.1071/AN12296

Totty VK, Greenwood SL, Bryant RH, Edwards GR 2013. Nitrogen partitioning and milk production of dairy cows grazing simple and diverse pastures. *Journal of Dairy Science 96*, 141-149.

Tozer KN, Cameron CA, Thom ER 2011. Pasture persistence: farmer observations and field measurements. *Pasture Persistence – Grassland Research and Practice Series 15*, 25-30.

Tozer KN, Minnee EMK, Greenfield RM, Cameron CA 2017. Effects of pasture base and species mix complexity on persistence and weed ingress in summer-dry dairy pastures. *Crop and Pasture Science 68*(6), 561-573. doi: https://doi.org/10.1071/CP17032

Valderrama X, Anrique R 2011. In situ rumen degradation kinetics of high-protein forage crops in temperate climates. *Chilean Journal of Agricultural Research 71*, 572-577.

Van Vuuren AM, Smits MCJ 1997. Effect of nitrogen and sodium chloride intake on production and composition of urine in dairy cows. In: Jarvis SC, Pain BF ed. Gaseous Nitrogen Emissions from Grasslands. Oxon, UK, CAB International. Pp. 95-99.

Verbic J, Chen XB, MacLeod NA, Ørskov ER 1990. Excretion of purine derivatives by ruminants. Effect of microbial nucleic acid infusion on purine derivative excretion by steers. *The Journal of Agricultural Science 114*(3), 243-248. doi: 10.1017/S0021859600072610

Vibart R, Tavendale M, Burke J, Pacheco D 2012. In vitro fermentation of [15N-] ryegrass and ruminal digesta of sheep grazing a ryegrass-based pasture in the morning or in the afternoon. *Proceedings of the New Zealand Society of Animal Production 72*, 100-105.

Vibart RE, Vogeler I, Dodd M, Koolaard J 2016. Simple versus Diverse Temperate Pastures: Aspects of Soil–Plant–Animal Interrelationships Central to Nitrogen Leaching Losses. *Agronomy Journal 108*(6), 2174-2188. doi: 10.2134/agronj2016.04.0193

Waghorn GC 2002. Can forages match concentrate diets for dairy production? *New Zealand Society of Animal Production 62*, 261-266.

Waghorn GC, Barry TNIe 1987. Pasture as a nutrient source. In: Nicol AM ed. Feeding livestock on pasture. Occasional publication No. 10, New Zealand Society of Animal Production. Pp. 21-38.

Waldrip HM, Todd RW, Cole NA 2013. Prediction of nitrogen excretion by beef cattle: a meta-analysis. *Journal of Animal Science 91*, 4290-4302.

Wales WJ, Heard JW, Ho CKMet al. 2006. Profitable feeding of dairy cows on irrigated dairy farms in northern Victoria. *Australian Journal of Experimental Agriculture 46*(7), 743-752. doi: https://doi.org/10.1071/EA05357

Webb CJ, Skyes WR, Garnock-Jones PJ 1988. Flora of New Zealand. 4 ed. Lincoln, Manaaki Whenua Press. 1365 p.

Whitehead DC 1995. Grassland Nitrogen. Wallingford, U.K., CAB International. 416 p.

Wilman D, Riley JA 1993. Potential nutritive value of a wide range of grassland species. *The Journal of Agricultural Science 120*(1), 43-50. doi: 10.1017/S0021859600073573

Wilman D, Derrick RW 1994. Concentration and availability to sheep of N, P, K, Ca, Mg and Na in chickweed, dandelion, dock, ribwort and spurrey, compared with perennial ryegrass. *Journal of Agricultural Science 122*(2), 217-223. doi: 10.1017/S0021859600087396

Woods R 2017. The effect of alternative forage species and gibberellic acid on nitrate leaching. Unpublished thesis, Lincoln University.

Woods RR, Cameron KC, Edwards GR, Di HJ, Clough TJ 2016. Effects of forage type and gibberellic acid on nitrate leaching losses. *Soil Use and Management 32*(4), 565–572.

Woodward SL, Laboyrie PJ, Jansen EBL 2000. Lotus corniculatus and condensed tannins - effects on milk production by dairy cows. *AsianAustralasian Journal of Animal Sciences 13*, 521-525.

Woodward SL, Waghorn GC, Bryant MA, Benton A 2012. Can diverse pasture mixtures reduce nitrogen losses? *Proceeding of the 5th Australasian Dairy Science Symposium*, 463-464.

Woodward SL, Waugh CD, Roach CG, Fynn D, Phillips J 2013. Are diverse species mixtures better pastures for dairy farming? *Proceedings of the New Zealand Grassland Association 75*, 79-84.

Zhou YC, Zheng RL 1991. Phenolic compounds and an analog as superoxide anion scavengers and antioxidants. *Biochem Pharmacol 42*(6), 1177-9.

Zhu H, Wang Y, Liu Zet al. 2016. Antidiabetic and antioxidant effects of catalpol extracted from Rehmannia glutinosa (Di Huang) on rat diabetes induced by streptozotocin and high-fat, high-sugar feed. *Chinese Medicine 11*(1), 25. doi: 10.1186/s13020-016-0096-7

Appendix A

Contrasts of data for Chapter 4

A.1 Contrasts of functional groups and forages within their groups from Chapter 4

Table A. 1 The effects of forage type, N fertiliser inputs (high vs low) and time of day (AM vs PM) on the crude protein concentration of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn).

	Autumn	Summer	Spring
Forage effects			
Grasses*Herbs	**<0.001**	**<0.001**	0.234
Herbs*Legumes	**<0.001**	**<0.001**	**<0.001**
Grasses*Legumes	**<0.001**	**<0.001**	**<0.001**
Per.RG*Itl.RG	**0.002**	**0.047**	0.057
White*Red	**<0.001**	**0.002**	0.126
Plantain*Chicory	**<0.001**	**<0.001**	**0.021**
N rate effects			
Grasses*Herbs	0.065	0.459	**0.013**
Herbs*Legumes	**0.042**	**<0.001**	0.121
Grasses*Legumes	**0.001**	**<0.001**	**0.002**
Per.RG*Itl.RG	0.325	0.396	0.428
White*Red	0.514	0.237	0.624
Plantain*Chicory	0.069	**0.044**	0.870
Time of day effects			
Grasses*Herbs	**<0.001**	**0.019**	**0.015**
Herbs*Legumes	0.085	0.091	0.337
Grasses*Legumes	**<0.001**	0.584	0.255
Per.RG*Itl.RG	**<0.001**	0.471	0.926
White*Red	0.653	0.969	0.642
Plantain*Chicory	0.187	0.055	0.058

Table A. 2 The effects of forage type, N fertiliser inputs (high vs low) and time of day (AM vs PM) on the water soluble carbohydrate concentration of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn).

	Autumn	Summer	Spring
Forage effects			
Grasses*Herbs	0.842	**<0.001**	**<0.001**
Herbs*Legumes	**<0.001**	**<0.001**	**<0.001**
Grasses*Legumes	**<0.001**	**<0.001**	**0.016**
Per.RG*Itl.RG	0.686	0.056	**<0.001**
White*Red	**<0.001**	0.154	0.109
Plantain*Chicory	**0.010**	**0.015**	0.566
N rate effects			
Grasses*Herbs	**0.003**	**<0.001**	**0.002**
Herbs*Legumes	0.741	0.761	0.803
Grasses*Legumes	**0.006**	**<0.001**	**0.006**
Per.RG*Itl.RG	**0.030**	**0.041**	0.047
White*Red	0.775	0.753	0.695
Plantain*Chicory	0.786	0.653	0.820
Time of day effects			
Grasses*Herbs	**<0.001**	**0.006**	**0.001**
Herbs*Legumes	**<0.001**	**0.005**	0.373
Grasses*Legumes	**<0.001**	**<0.001**	**<0.001**
Per.RG*Itl.RG	**0.001**	0.360	0.959
White*Red	0.256	0.300	0.103
Plantain*Chicory	0.064	**0.022**	**0.038**

Table A. 3 The effects of forage type, N fertiliser inputs (high vs low) and time of day (AM vs PM) on the neutral detergent fibre concentration of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn).

	Autumn	Summer	Spring
Forage effects	**<0.001**	**<0.001**	**<0.001**
Grasses*Herbs	**<0.001**	0.084	**<0.001**
Herbs*Legumes	**<0.001**	**<0.001**	**<0.001**
Grasses*Legumes	**<0.001**	0.873	**<0.001**
Per.RG*Itl.RG	**<0.001**	**0.020**	**<0.001**
White*Red	**<0.001**	**<0.001**	**<0.001**
Plantain*Chicory			
N rate effects	**<0.001**	0.388	**0.002**
Grasses*Herbs	0.197	0.459	**0.018**
Herbs*Legumes	**0.003**	0.950	0.639
Grasses*Legumes	0.457	0.390	**0.018**
Per.RG*Itl.RG	0.350	0.970	0.390
White*Red	0.249	0.464	0.164
Plantain*Chicory			
Time of day effects	**<0.001**	**<0.001**	**0.040**
Grasses*Herbs	0.862	0.108	**0.001**
Herbs*Legumes	**<0.001**	**0.004**	0.063
Grasses*Legumes	**<0.001**	0.549	0.953
Per.RG*Itl.RG	0.318	0.767	0.794
White*Red	0.055	0.975	0.997
Plantain*Chicory	**<0.001**	**<0.001**	**<0.001**

Table A. 4 The effects of forage type, N fertiliser inputs (high vs low) and time of day (AM vs PM) on the DOMD concentration of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn).

	Autumn	Summer	Spring
Forage effects			
Grasses*Herbs	**<0.001**	0.449	0.331
Herbs*Legumes	**<0.001**	**<0.001**	0.082
Grasses*Legumes	**<0.001**	**0.002**	**0.020**
Per.RG*Itl.RG	**<0.001**	0.081	**0.034**
White*Red	**<0.001**	**<0.001**	**<0.001**
Plantain*Chicory	**<0.001**	**<0.001**	**<0.001**
N rate effects			
Grasses*Herbs	0.576	**0.026**	**0.011**
Herbs*Legumes	0.875	0.169	0.637
Grasses*Legumes	0.686	0.390	0.071
Per.RG*Itl.RG	0.137	0.259	0.073
White*Red	0.260	0.876	0.756
Plantain*Chicory	0.856	0.359	0.371
Time of day effects			
Grasses*Herbs	**<0.001**	**0.002**	**0.006**
Herbs*Legumes	0.088	0.286	0.125
Grasses*Legumes	**<0.001**	**0.043**	0.383
Per.RG*Itl.RG	**<0.001**	0.623	0.539
White*Red	0.474	0.153	0.686
Plantain*Chicory	**0.037**	0.326	0.417

Appendix B

Harvest dates of forages

B.1 Harvest dates of forages for nutritive value analysis in Chapter 4

Table A.5 Harvest dates of grasses and herbs and legumes for each season

Group	Season	Harvest date
Grasses and herbs	Spring	8/10/2014
		29/10/2014
	Summer	24/11/2014
		16/12/2014
		13/01/2015
		10/02/2015
	Autumn	10/03/2015
		3/04/2015
		18/05/2015
Legumes	Spring	23/10/2014
	Summer	24/11/2014
		30/12/2014
		3/02/2015
	Autumn	10/03/2015
		12/04/2015
		2/06/2015

Appendix C

Harvest dates of plantain

C.1 Harvest dates of plantain

Table A. 6 Harvest dates of plantain for secondary plant compound analysis in Chapter 7

Year	Season	Harvest date
1	Spring	29/10/2014
	Summer	13/01/2015
	Autumn	3/04/2015
2	Spring	6/10/2015
	Summer	2/02/2016
	Autumn	31/03/2016

Appendix D

Refereed Publications during the course of study

D.1 **Box, L. A.,** Edwards, G. R., & Bryant, R. H. 2016. Milk production and urinary nitrogen excretion of dairy cows grazing perennial ryegrass-white clover and pure plantain pastures. *Proceedings of the New Zealand Society of Animal Production 76*, 18-21.

D.2 **Box, L. A.,** Edwards, G. R., & Bryant, R. H. 2017. Milk production and urinary nitrogen excretion of dairy cows grazing plantain in early and late lactation. *New Zealand Journal of Agricultural Research 60*(4):470-482 doi:10.1080/00288233.2017.1366924

D.3 **Box, L. A.**, Edwards, G. R., & Bryant, R. H. 2017. Diurnal changes in the nutritive composition of four forage species at high and low N fertiliser. *Journal of New Zealand Grasslands 79*, 43-50.

D.4 **Box, L. A.,** Edwards, G. R., & Bryant, R. H. 2018. *In sacco* digestion kinetics of plantain and ryegrass-white clover harvested in the morning and afternoon. *New Zealand Journal of Animal Science and Production 78*, 34-39.

D.5 **Box, L. A.**, Edwards, G. R., & Bryant, R. H. 2018. Seasonal and diurnal changes in plantain secondary compounds grown at high and low N inputs. *New Zealand Journal of Agricultural Research*, doi:10.1080/00288233.2018.1505641

Submitted publications during the course of study

D.6 **Box, L. A.**, Edwards, G. R., & Bryant, R. H. (submitted 2017). Milk production from late lactation cows grazing temporally and spatially separated monocultures of plantain and ryegrass-white clover. *Animal*

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

LA Box*, GR Edwards and RH Bryant

Faculty of Agriculture and Life Sciences, PO Box 85084, Lincoln University, Lincoln 7647, Christchurch

**Corresponding author*: Email: lisa.box@lincolnuni.ac.nz

Abstract

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

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

Introduction

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

The objective of this experiment was to compare milk production and urinary N concentration of late-lactation dairy cows offered perennial ryegrass-white clover and pure plantain pastures in autumn.

Materials and methods

Experimental site and treatments

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

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

Herbage measurements

At least 50 compressed pasture height measurements were taken daily pre- and post-grazing using a calibrated rising plate meter (RPM; Jenquip, Filip's EC 09, Electronic Folder plate meter). The pre-grazing measurements were taken in the area expected to be allocated in the next forage allocation. Calibration measurements were collected from pastures every second day by cutting two 0.2 m^2 quadrats to ground level before and after grazing during the experiment. Two RPM measurements were recorded in each quadrat prior to harvesting herbage. Collected herbage samples were weighed fresh and a subsample taken to determine botanical composition of pastures. All botanical components and bulk samples were oven dried at 65°C for 48 h and total dry weight and DM% determined. Linear and curvilinear relationships between herbage mass and pasture height were compared and best-fit equations (greatest r^2) were fitted to the data. The calibration equations for each pasture type were: perennial ryegrass-white clover (kg DM/ha) = 145 × height + 3.92, $r^2 = 0.85$, $P>0.001$, and plantain (kg DM/ha) = 73 × height + 248, $r^2 = 0.89$, $P>0.001$. Using equations derived from experimental data set and grazing areas, the actual daily herbage allocation was calculated as 21.4 ± 2.4 kg/DM/cow for pasture, 19.1 ± 0.94 kg/DM/cow for plantain and 22.2 ± 0.9 kg/DM/cow for 50-50 pasture-plantain. Apparent group DM intake of cows was calculated from herbage disappearance between pre- and post-grazing calibrated RPM measurements and areas allocated.

Subsampled herbage was sorted into sown grass, white clover, weeds and dead material for perennial ryegrass-white clover pastures and plantain, weeds and dead material for plantain pastures. Sorted material and a bulk sample of the pasture was oven dried at 65°C for 48 h and dry weight recorded to determine DM/ha and botanical composition of pastures. Bulk dried samples were ground through a 1-mm sieve and scanned by near infra-red spectrophotometer (NIRS, NIRSystems 5000, Foss, Maryland, USA) to determine crude protein (CP), digestible organic matter (DOMD), water-soluble carbohydrate (WSC), acid-detergent fibre and neutral-detergent fibre (NDF). Metabolisable energy (ME) was calculated as MJ ME/kg DM = 0.16 × DOMD (CSIRO 2007).

Animal measurements

Milk yield was measure daily for individual cows with an automated system (Alpro Herd management system, Tumba Sweden). Two milk samples were collected every second day for every cow in the afternoon and following morning milking to determine milk composition and milk urea nitrogen (MUN). The sample for milk composition was analysed by Livestock Improvement Corporation Ltd (Christchurch, New Zealand) to determine milk fat, protein and lactose by MilkoScan (Foss Electric, Hillerod, Denmark). The second sample for MUN was centrifuged at 4,000 × g for 10 min at room temperature and refrigerated for 10 min to allow the fat to solidify on the top and be removed. Skim milk was pipetted into a clean micro-centrifuge tube and frozen before analysis. Skim milk was analysed by Lincoln University Analytical Services for MUN by automated Modular P analyser (Roche/Hitachi).

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

Statistical analysis

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

Results

Herbage

Herbage characteristics and composition for plantain and ryegrass-white clover pastures are shown in Table 1. Post-grazing herbage mass was lower for plantain than ryegrass pastures (Table 1). ME and CP was similar across pasture treatments (range of 10.9 to 11.4 and 21.5 to 23.3, respectively). Water soluble carbohydrate (WSC) concentration was in higher in plantain than pasture.

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

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

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

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

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

	Pasture	Plantain	50-50 pasture-plantain	LSD	P value
Urine					
NH3 (mmol/L)	1.98a	0.97c	1.35b	0.30	<0.001
Urea (mmol/L)	144.3a	61.5c	94.8b	15.8	<0.001
N concentration (g N/L)	5.4a	2.4c	3.6b	0.06	<0.001
Creatinine (mmol/L)	1.67a	0.88c	1.27b	0.24	<0.001
Faeces					
N (%)	3.43	3.45	3.33	0.11	0.070
DM (%)	10.9a	15.7c	12.6b	0.86	<0.001

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

Animal

Milk volume (L) was higher for plantain than pasture (Table 2). Milksolids yield was greatest for plantain intermediate for 50-50 pasture-plantain and lowest for cows grazing pasture (Table2). There was no significant difference in the milk protein percent among treatments (Table 2). Milk fat percent was lower for 50-50 pasture-plantain than for pasture (Table 2). Cows grazing plantain and 50-50 pasture-plantain produced more milk protein than those grazing pasture. Milk urea N was lowest for plantain. The concentration of NH_3, urea and N in urine was lowest for plantain, intermediate for 50-50 pasture-plantain and highest in pasture (Table 3). The concentration of N in the faeces was similar among treatments.

Discussion

Milksolid production per cow was greatest for cows grazing plantain (1.67 kg) compared to pasture (1.50 kg) with 50-50 pasture-plantain intermediate (1.6 kg). This was largely due to an increase in milk volume, by cows grazing plantain or 50-50 pasture-plantain. Milk protein percentage was unaffected by pasture treatment and fat percentage tended to be lower where plantain was included in the diet. A possible explanation for the increase in milk volume was the increase in apparent DMI for cows on plantain. This occurred because plantain was grazed to a lower post-grazing herbage mass compared with pasture despite, similar daily allocations of pasture. However, it is noteworthy that cows grazing 50-50 pasture-plantain had the highest apparent DMI and this did not translate to increased milk production.

Previous short term experiments have shown that when plantain is included in mixtures with ryegrass, white clover and chicory, urinary N concentration of dairy cows were reduced by at least 20% compared with perennial ryegrass-white clover pastures (Woodward et al. 2012; Totty et al. 2013). In the current experiment, urinary N concentration was 50% and 33% lower, respectively for plantain and 50-50 pasture-plantain, compared with pasture. The MUN concentration, an indicator of surplus dietary N (Cosgrove et al. 2014), was also lower in plantain than pasture or 50-50 pasture-plantain. Previous studies have shown the excretion of N in urine to be linearly related to N intake (Tas et al. 2006; Higgs et al. 2012). In this experiment the difference in apparent N intake between pasture and plantain was small at 11 g N/cow/day, and is unlikely to be sufficient to explain the large (3 g N/L) difference in urine N concentration. It is possible that the higher WSC concentration of plantain compare to pasture (10.9 vs 7.61 g/kg DM) contributed to the lower urine N concentration of urine from cows with plantain (Edwards et al. 2007), although values were low (>10%) and are unlikely to explain differences in high CP (>21%) forages seen in this study. Another explanation for lower urine N concentration is a greater urine volume from cows grazing plantain as a consequence of with higher mineral content (Ledgard et al. 2015), secondary plant compounds or increased water intake due to a lower DM% of plantain. Some indication of this is the 47% decrease in creatinine, a marker of urine volume (Chizzotti et al. 2008; Waldrip et al. 2013), for cows grazing plantain compared with pasture.

Conclusions

The results demonstrate a role for the use plantain as a mitigation strategy to reduce the environmental impact of dairy farming. By providing plantain as a monoculture or with perennial ryegrass-white clover pastures to cows in late lactation, milksolids production was increased and urine N concentration was reduced. The decline in urine N concentration may decrease N loading from the urine patch and reduce the risk of nitrate leaching for dairy grazing systems.

References

Bryant JR, Hoogendoorn CJ, Snow VO 2007. Simulation of mitigation strategies to reduce nitrogen leaching from grazed pasture. Proceedings of the New Zealand Grassland Association 69: 145-151.

Chizzotti ML, Valadares Filho SC, Valadares RFD, Chizzotti FHM, Tedeschi LO 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. Livestock Science 113: 218-225.

Cosgrove GP, Taylor PS, Lowe KA, Foote AG, Jonker A 2014. Milk urea estimates of nitrogen excretion by dairy cows grazing forage species with contrasting chemical and morphological characteristics. Proceedings of the 5th Australasian Dairy Science Symposium: 249-251.

CSIRO 2007. Nutrient requirements of domesticatede ruminants. Eds. Freer M, Dove H, Nolan JV. Collingwood Australisa. pp 8.

Di HJ, Cameron KC 2007. Nitrate leaching losses and pasture yields as affected by different rates of animal urine nitrogen returns and application of a nitrification inhibitor - a lysimeter study. Nutrient Cycling and Agroecosystems 79: 281-290.

Edwards GR, Parsons AJ, Rasmussen S 2007. High sugar ryegrasses for dairy systems. Meeting the Challenges for Pasture-Based Dairying: 307-334.

Edwards GR, Bryant RH, Smith N, Hague H, Taylor S, Ferris A, Farrell L 2015. Milk production and urination behaviour of dairy cows grazing diverse and simple pastures. Proceedings of the New Zealand Society of Animal Production 75: 79-83.

Haultain J, Wigley K, Lee JM 2014. Rising plate meters and a capacitance probe estimate the biomass of chicory and plantain monocultures with similar accuracy as for ryegrass-based pasture. Proceedings of the New Zealand Grassland Association 76: 67-74.

Higgs RJ, Chase LE, van Amburgh ME 2012. Development and evaluation of equation sin the Cornell Net Carbohydrate and Protein System to predict nitrogen excretion in lactating dairy cows. Journal of Dairy Science 95: 2004-2014.

Ledgard SF, Welten B, Betteridge K 2015. Salt as a mitigation option for decreasing nitorgen leaching losses from grazed pastures. Journal of the Science of Food and Agriculture 95(15): 3033–3040.

Miller M, Bryant RH, Edwards GR 2012. Dry matter intake and nitrogen losses of pregnant, non-lactating dairy cows fed kale with a range of supplements in winter. Proceedings of the New Zealand Society of Animal Production 72: 8-13.

Tamminga S 1992. Nutrition management of dairy cows as a contribution of pollution control. Journal of Dairy Science 75: 345-357.

Tas BM, Taweel HZ, Smit HJ, Elgersma A, Dijkstra J, Tamminga S 2006. Utilisation of N in perennial ryegrass cultivars by stall-fed lactating dairy cows. Livestock Science 100: 159-168.

Totty VK, Greenwood SL, Bryant RH, Edwards GR 2013. Nitrogen partitioning and milk production of dairy cows grazing simple and diverse pastures. Journal of Dairy Science 96: 141-149.

Waldrip HM, Todd RW, Cole NA 2013. Prediction of nitrogen excretion by beef cattle: a meta-analysis. Journal of Animal Science 91: 4290-4302.

Woodward SL, Waghorn GC, Bryant MA, Benton A 2012. Can diverse pasture mixtures reduce nitrogen losses? Proceeding of the 5th Australasian Dairy Science Symposium: 463-464.

NEW ZEALAND JOURNAL OF AGRICULTURAL RESEARCH, 2017
VOL. 60, NO. 4, 470–482
https://doi.org/10.1080/00288233.2017.1366924

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Milk production and urinary nitrogen excretion of dairy cows grazing plantain in early and late lactation

Lisa A. Box, Grant R. Edwards and Racheal H. Bryant

Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln, New Zealand

ABSTRACT

The effects of 50% or 100% of a herbage diet of plantain on milk production and urinary nitrogen (N) concentration were measured in two experiments for late (autumn 2015) and early (spring 2015) lactation dairy cows. Three groups of 12 mixed age Friesian × Jersey dairy cows were offered a perennial ryegrass-white clover pasture, or pure plantain or 50% perennial ryegrass-white clover and 50% pure plantain by ground area (50–50 pasture–plantain). Urine N concentration was lower in both experiments ($P < .001$) for plantain (2.4 and 2.2 g N/L) and 50–50 pasture–plantain (3.6 and 3.4 g N/L) than pasture (5.4 and 4.7 g N/L). Cows on plantain or 50–50 pasture-plantain produced at least as much milk as those on pasture in both experiments. Plantain may offer environmental benefits to dairy systems by reducing the N concentration of urine deposited on the soil from grazing cows.

ARTICLE HISTORY
Received 1 June 2017
Accepted 10 August 2017

KEYWORDS
Herb; *Lolium perenne*; nitrate; *Plantago lanceolata*; *Trifolium repens*

Introduction

Reducing the environmental impact of the dairy industry in New Zealand has become a key focus with an increasing interest in water quality issues, in particular nitrogen (N) leaching from pastoral land (Bryant et al. 2007; Woodward et al. 2012). Regional Councils throughout New Zealand have been developing regulations that place a limit on the amount of nitrate-N leached from agricultural land. Nitrogen from urine patches is a major contributor to N leaching (Di and Cameron 2007). There is a large discrepancy between the N content of grazed forages, and the N requirement of the animals; N in excess of an animal's requirement is excreted, primarily in the urine (Tamminga 1992). This leads to high N loading (up to 1000 kg N/ha) on the soil at the urine patch, which is easily leached when drainage occurs (Di and Cameron 2007). Mitigation strategies have utilised variations in the chemical composition (water-soluble carbohydrate (WSC) and crude protein (CP)), mineral profile and secondary plant compounds in forages, to reduce urinary N excretion or divert dietary N away from urine. Previous experiments (Woodward et al. 2012; Totty et al. 2013; Edwards et al. 2015; Bryant et al. 2017) achieved lower urinary N from cows by incorporating alternative pastures species including plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*) and additional legumes such as red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*)

CONTACT Lisa A. Box ✉ lisa.box@lincolnuni.ac.nz

into pasture mixtures with perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). In comparison with standard perennial ryegrass-white clover pastures, these diverse mixtures have shown consistent reductions in urinary N concentration of at least 20% while maintaining or improving milk production (Woodward et al. 2012; Totty et al. 2013; Bryant et al. 2017). However, it is unclear whether the effects are due to an individual species in the mixture rather than the diversity. Research using sheep fed plantain or perennial ryegrass diets indicated a positive relationship between plantain feeding and urine volume (O'Connell et al. 2016) which may explain the differences in urine N concentration observed in dairy cows. There is currently little research comparing perennial ryegrass pasture with plantain forage fed as a sole diet. The objective of this study was to assess whether plantain as a grazed pasture species can support milk production similar to that achieved with standard ryegrass-white clover while reducing urinary N losses. We hypothesise that cows grazing plantain or a 50–50 pasture–plantain diet will produce as much milk as those on pasture with lower urinary N concentrations.

Materials and methods

Experimental site and treatments

Two experiments were conducted at the Lincoln University Research Dairy Farm (LURDF) with the approval of the Lincoln University Animal Ethics Committee in late (AEC 592) and early (AEC 639) lactation in 2015. There were three pasture treatments: (1) a perennial ryegrass-white clover pasture ($n = 12$ cows); (2) plantain ($n = 12$ cows) and (3) an area that was made of 50% perennial ryegrass-white clover and 50% pure plantain by ground area ($n = 12$ cows). A group of 36 pregnant, lactating Friesian × Jersey dairy cows were blocked into three groups of 12 cows according to milk solids production (1.31 ± 0.03 kg MS/cow/day for late and 2.48 ± 0.04 kg MS/cow/day for early lactation), age (5.9 ± 0.4 years for late and 5.6 ± 0.3 years for the early lactation experiment), days in milk (217 ± 3.2 days for the late and 50.9 ± 1.1 days for the early lactation experiments) and liveweight (515 ± 8.6 kg for the late and 496 ± 8.4 kg for the early lactation experiments) (all mean ± SEM). Both studies were conducted over a 10-day period with late lactation occurring from 19 to 29 March 2015 and the early lactation experiment occurring from 8 to 18 October 2015. This included a 3-day transition period where cows grazed their respective pasture treatments with no measurements and a 7-day experimental period. Before the experiments, all cows grazed perennial ryegrass-white clover pasture together. Cows were milked twice daily at approximately 0600 and 1400 h.

The perennial ryegrass (cv. Arrow)-white clover (cv. Kopu II) pastures were established across one-half of three 1.5 ha paddocks in March 2014. In December 2014, the remaining half of the same three paddocks were sown in plantain (cv. Tonic). Paddocks were grazed with dairy cows rotationally to a constant height of approximately 1500 kg DM/ha and fertilised with urea at a rate of 70 kg N/ha 25 days prior to the experiment. Pasture was irrigated with a centre-pivot irrigator. Each 1.5 ha paddock was separated into the three forage treatments, of pasture only, a 50–50 pasture–plantain and pure plantain area, using electric fencing. Due to low pasture growth rates in the late lactation study, more area was required to meet feed allocation targets so an additional perennial ryegrass and white clover pasture paddock was included for the 50–50 pasture–plantain treatment.

After each morning milking, cows were offered a target daily allowance of 18 kg DM/cow/day above 3.5 cm in the late lactation experiment and 30 kg DM/cow/day above ground level in the early lactation experiment. Daily herbage allocation during the experiment was based on a national calibration equation for perennial ryegrass-white clover (kg DM/ha = 140 × Rising Plate Meter (RPM) reading + 150) and previously derived calibration equations between herbage mass and pasture height for plantain pastures (kg DM/ha = 94 × RPM reading + 455) (Haultain et al. 2014). For the 50–50 pasture–plantain treatment pre-grazing plate meter readings were taken in both the pasture and plantain areas. The sum of 0.5 × pasture herbage mass and 0.5 × plantain herbage mass was used to determine the area to be grazed as spatially separated pasture and plantain. Daily allocated areas were controlled by temporary electric fencing. Back fencing was used to prevent grazing of residual regrowth. Cows had *ad lib* access to water through a portable trough.

Herbage measurements

At least 50 compressed pasture height measurements were recorded daily pre- and post-grazing using a calibrated rising plate meter (RPM; Jenquip, Filip's EC 09, Electronic Folding plate meter). The pre-grazing measurements were recorded in the area estimated to be allocated in the next forage allocation. Calibration measurements were collected from pastures every second day by cutting two 0.2 m^2 quadrats to ground level in late lactation and five in early lactation before and after grazing during each experiment. Two RPM measurements were recorded in each quadrat prior to harvesting herbage. Collected herbage samples were weighed fresh and a subsample taken to determine botanical composition of pastures. All botanical components and bulk samples were oven dried at 65°C for 48 h and total dry weight and DM% determined. Linear and curvilinear relationships between herbage mass and pasture height were compared and best-fit equations (greatest r^2) were fitted to the data. The calibration equations for each pasture type were: ryegrass-clover (kg DM/ha) = 145 × height (RPM clicks) + 3.92, $r^2 = 0.85$ and plantain (kg DM/ha) = 73 × height (RPM clicks) + 248, $r^2 = 0.89$. In the late lactation experiment, calibration equations were: pasture (kg DM/ha) = 120 × height (RPM clicks) + 312, $r^2 = 0.63$ and plantain (kg DM/ha) = 114 × height (RPM clicks) + 470, $r^2 = 0.71$. Using equations derived from experimental data sets and grazing areas, the actual daily herbage allocation was calculated as 21.4 ± 2.4 kg/DM/cow for pasture, 19.1 ± 0.94 kg/DM/cow for plantain and 22.2 ± 0.9 kg/DM/cow for 50–50 pasture–plantain in late lactation. In the early lactation experiment, actual daily herbage allocation was calculated as 32.8 ± 1.3 kg/DM/cow for pasture, 31.3 ± 0.65 kg/DM/cow for plantain and 31.6 ± 0.37 kg/DM/cow for 50–50 pasture–plantain. Apparent group DM intake of cows was calculated from herbage disappearance between pre- and post-grazing calibrated RPM measurements and areas allocated.

Subsampled herbage was manually sorted into sown species, weeds and dead material. Sorted material and a bulk sample of the pasture was oven dried at 65°C for 48 h to determine botanical composition of pastures. Bulk oven-dried samples were ground through a

1-mm sieve and scanned by a near infra-red spectrophotometer (NIRS, NIRSystems 5000, Foss, Maryland, USA) to determine CP, digestible organic matter in the dry matter (DOMD), WSC (including soluble starch), acid detergent fibre (ADF) and neutral-detergent fibre (NDF). Calibration equations for NIRS were derived from perennial ryegrass, clover and plantain herbage. Ground samples were then subsampled and analysed for mineral composition by inductively coupled plasma atomic emission spectroscopy. Plantain samples were also analysed for secondary plant compounds (catalpol, aucubin and acteoside) using high performance liquid chromatography by Massey University analytical laboratory following procedures outlined by Tamura and Nibishe (2002). Metabolisable energy (ME) was calculated as MJ ME/kg DM = 0.16 × DOMD (CSIRO 2007).

Animal measurements

Milk yield was measured daily for individual cows with an automated system (DeLavel Alpro Herd Management System, DeLavel, Tumba, Sweden). Milk samples from all cows were collected on days 4, 6, 8 and 10 from the afternoon and following morning milking to determine milk composition and milk urea nitrogen (MUN). Fat, protein and lactose composition in milk were analysed by Livestock Improvement Corporation Ltd (Christchurch, New Zealand) by MilkoScan (Foss Electric, Hillerod, Denmark). Samples for MUN were centrifuged at 4000*g* for 10 min at room temperature and refrigerated for 10 min to allow the fat to solidify on the top and be removed. Skim milk was pipetted into a clean micro-centrifuge tube, and frozen for later analysis of thawed samples using an automated Modular P analyser (Roche/Hitachi).

Spot samples of urine and faeces were collected on days 5, 7 and 9 of the experiments from all cows following morning and afternoon milking. Spot urine samples were collected mid-stream by vulval stimulation and were immediately acidified with hydrochloric acid to prevent volatilisation and stored at −20°C. Faeces were obtained from voluntary events in the yards or by rectal stimulation and stored at −20°C. Faeces were freeze dried and ground through a 1-mm sieve. Faecal N content was determined by combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH, Hanau, Germany). Urine N content of thawed subsamples was determined by an autoanalyser (Roche Cobas Mira Plus CC, Minnesota, USA). Urea content of acidified urine was analysed using a commercial enzymatic kinetic technique (Randox, Crumlin, Co., Antrim, UK). Creatinine content of acidified urine was determined calorimetrically using a commercial kit from Randox laboratories (Crumlin, Co., Antrim, UK).

A urine meter harness was worn by all cows for up to 24 h between days 5 and 10 for both experiments. The harness involved attaching a flow meter to the vulva of the cow which transmits pulse signals, whenever the cow urinates, to a data logger carried in a cover worn by the cow (Ravera et al. 2015). On occasions, the urine meter would fail through becoming unstuck, wire breakage or sensor failure. This left 20 cows in the late lactation experiment (six from pasture, seven from 50–50 pasture–plantain and seven from plantain) from which data were used and 22 cows in the early lactation experiment (eight from pasture, seven from 50–50 pasture–plantain and seven from plantain).

Urinary N output was estimated using three methods: (1) urine volumes measured using the urine harness were multiplied by overall treatment urine N concentration averages, (2) creatinine-based equation: urinary N (g/day) = (21.9 (mg/kg) × LW (kg) ×

(1/urinary creatinine (mg/kg))) × urine N (g/kg) (Pacheco et al. 2007) and (3) MUN-based equation: urinary N (g/day) = 0.026 × LW (kg) × milk urea N (mg/dL) (Jonker et al. 1998).

The effect of treatment on rumen microbial activity was calculated from the ratio of purine derivatives (PD) to creatinine in spot samples of urine (Chen 1989; Verbic et al. 1990; Chen et al. 1995). The resulting PD index is a relative measure of microbial protein synthesis:

$$\text{PD index} = \left\{\frac{[\text{total PD}\,(\text{mmol/L})]}{\text{creatinine}\,(\text{mmol/L})}\right\} \times BW^{0.75},$$

where total PD [allantoin (mmol/L) + uric acid (mmol/L)].

Statistical analysis

Treatment means for milk, urine and faeces were determined using data from individual measurements from animal samples over sampling days. The effect of pasture type on milk, urine and faecal measurements was analysed for variance using GenStat 15.1, with cows as random effect and pasture type as fixed effect using a one-way ANOVA. Results were declared to be significant at $P < .05$. Means were separated using Fisher's protected least significance difference test. No statistical analysis was carried out on herbage samples or apparent DM intake as they were based on samples collected from each daily allocation within the same paddock. Intake and herbage measurements were estimated as means for the treatment group as animals grazed together in their treatment groups.

Results

Herbage

Herbage characteristics and composition of plantain and ryegrass-white clover pastures are shown in Table 1. In the late lactation experiment, pre-grazing mass of the pasture portion of the 50–50 pasture–plantain was 700 kg DM/ha greater than the herbage mass for any other treatment. Dry matter content of plantain was nearly half that of ryegrass in the late lactation experiment and about 20% lower in the early lactation experiment. Organic matter content was lower in plantain than in ryegrass in the late lactation experiment only. The mineral composition of pasture and plantain is shown in Table 2.

The post-grazing mass of plantain appeared to be lower than that of pasture in both experiments (Table 1). The apparent DM intake increased by about 20% for cows grazing plantain and 50–50 pasture–plantain compared with those grazing pasture (Table 3). In the early lactation experiment, apparent DM intake was greater for the plantain treatment only. The ME and CP was similar across pasture treatments for both experiments (Table 1). Similarly, apparent N intake and ME intake did not differ across treatments.

Total average water intake was similar for all treatments (150 L/cow/day) and more than two-thirds of total water were ingested in feed. As plantain intake increased, water intake from feed increased and drinking water declined (Table 3).

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

		50–50 pasture–plantain		
	Pasture	Pasture	Plantain	Plantain
Late lactation 2015				
Pre-grazing				
Herbage mass (kg DM/ha)	3209 ± 30.5	3935 ± 151	3005 ± 150	3187 ± 43.6
N%	3.7 ± 1.8	3.4 ± 1.0	3.7 ± 0.9	3.6 ± 0.3
ME (MJ ME/kg DM)	11.2 ± 0.1	10.9 ± 0.1	11.3 ± 0.1	11.4 ± 0.2
ADF (g/kg DM)	28.2 ± 1.0	29.5 ± 0.4	21.0 ± 0.3	22.4 ± 1.3
WSC (g/kg DM)	7.6 ± 0.7	5.3 ± 1.3	12.6 ± 0.7	10.9 ± 1.6
NDF (g/kg DM)	42.9 ± 2.2	49.1 ± 1.3	27.1 ± 0.7	29.9 ± 3.0
CP (g/kg DM)	23.3 ± 0.9	21.5 ± 0.6	22.9 ± 0.5	22.3 ± 0.6
Plantain (%)	0 ± 0	0 ± 0	89.7 ± 2.1	89.6 ± 2.5
Grass (%)	48.4 ± 5.0	58.8 ± 4.0	0 ± 0	0 ± 0
Legume (%)	25.7 ± 3.0	15.1 ± 3.2	0 ± 0	0 ± 0
Weed (%)	3.8 ± 3.0	0.7 ± 0.3	3.7 ± 1.0	6.1 ± 1.8
Dead (%)	22.1 ± 3.8	25.4 ± 4.3	6.6 ± 2.2	4.3 ± 1.1
DM (%)	17.1 ± 1.1	16.8 ± 1.1	10.7 ± 0.4	9.8 ± 0.3
Post-grazing				
Herbage mass (kg DM/ha)	1187 ± 11.3	1024 ± 28.6	638 ± 18.4	611 ± 22.7
Early lactation 2015				
Pre-grazing				
Herbage mass (kg DM/ha)	3705 ± 222	3886 ± 70.9	3611 ± 63.5	3800 ± 110
N%	2.98 ± 0.113	2.69 ± 0.115	3.25 ± 0.171	3.12 ± 0.136
ME (MJ ME/kg DM)	11.7 ± 0.123	11.7 ± 0.117	12.1 ± 0.156	11.8 ± 0.071
ADF (g/kg DM)	26.5 ± 0.623	26.6 ± 0.527	23.0 ± 0.713	24.7 ± 0.376
WSC (g/kg DM)	14.1 ± 1.56	15.3 ± 1.26	6.02 ± 0.551	6.07 ± 0.275
NDF (g/kg DM)	46.0 ± 1.90	46.9 ± 1.14	32.8 ± 1.32	34.0 ± 0.226
CP (g/kg DM)	18.6 ± 0.706	16.8 ± 0.716	20.3 ± 1.07	19.5 ± 0.852
Plantain (%)	0 ± 0	0.600 ± 0.4073	66.7 ± 3.70	67.8 ± 3.52
Grass (%)	74.7 ± 3.24	74.3 ± 2.08	0 ± 0	0 ± 0
Legume (%)	7.28 ± 1.911	7.90 ± 1.36	0 ± 0	0 ± 0
Weed (%)	3.90 ± 0.927	1.66 ± 0.578	25.3 ± 3.46	20.8 ± 2.78
Dead (%)	14.2 ± 2.74	15.5 ± 1.16	7.98 ± 2.366	11.4 ± 2.19
DM (%)	17.1 ± 0.67	18.0 ± 0.62	14.8 ± 0.60	14.3 ± 0.48
Post-grazing				
Herbage mass (kg DM/ha)	1579 ± 67.3	1664 ± 65.4	1437 ± 62.7	1281 ± 46.4

The mineral concentration was similar for pasture and plantain except for sodium and calcium. Sodium concentration was two times greater in plantain than pasture in the late lactation experiment only. Calcium concentration of the herbage was about three times

Table 2. Apparent intake of dairy cows grazing pasture, plantain or 50–50 pasture–plantain in autumn and spring and water intake in spring (±SEM).

	Pasture	50–50 pasture–plantain	Plantain
Late lactation 2015			
DMI	13.5 ± 0.54	14.9 ± 0.48	15.9 ± 0.51
MJ ME/cow/day	173 ± 8.1	182 ± 10.0	192 ± 11.7
Average of N intake/cow/day	619 ± 62.6	553 ± 13.7	594 ± 20.4
Early lactation 2015			
DMI	18.5 ± 0.38	18.5 ± 0.43	20.7 ± 0.70
MJ ME/cow/day	234 ± 10.1	245 ± 6.93	275 ± 10.8
Average of N intake/cow/day	652 ± 4.40	629 ± 3.31	669 ± 4.80
Water intake			
From trough	40.3 ± 2.82	22.8 ± 2.15	10.0 ± 1.48
From herbage	109 ± 12.8	115 ± 7.3	151 ± 9.8
Total	147 ± 10.6	140 ± 14.2	163 ± 11.5

Table 3. Mean herbage mineral composition (% DM) bioactive glycoside (mg/g dry DM) (±SEM) of pasture, plantain or 50–50 pasture–plantain sampled to ground level.

		50–50 pasture–plantain		
	Pasture	Pasture	Plantain	Plantain
Late lactation 2015				
Sodium	0.380 ± 0.054	0.420 ± 0.089	0.965 ± 0.100	0.985 ± 0.099
Magnesium	0.247 ± 0.020	0.250 ± 0.005	0.197 ± 0.007	0.183 ± 0.017
Calcium	0.807 ± 0.072	0.583 ± 0.039	2.26 ± 0.096	2.13 ± 0.033
Chloride	1.58 ± 0.110	1.52 ± 0.062	2.07 ± 0.545	2.04 ± 0.234
Sulphur	0.270 ± 0.029	0.260 ± 0.024	0.367 ± 0.041	0.327 ± 0.010
Potassium	3.47 ± 0.268	3.40 ± 0.287	3.53 ± 0.288	3.13 ± 0.378
Phosphorus	0.363 ± 0.019	0.383 ± 0.024	0.410 ± 0.017	0.383 ± 0.007
DCAD	441 ± 43.0	461 ± 48.7	513 ± 108	448 ± 36.5
K/Na Ratio	9.33 ± 1.089	10.33 ± 3.569	3.67 ± 0.272	3.33 ± 0.720
Catalpol			0.063 ± 0.022	0.088 ± 0.010
Aucubin			5.67 ± 0.763	4.66 ± 0.355
Acteoside			9.29 ± 1.85	11.3 ± 0.695
Early lactation 2015				
Sodium	0.691 0.118	0.894 ± 0.107	1.07 ± 0.135	1.10 ± 0.174
Magnesium	0.193 ± 0.10	0.183 ± 0.003	0.183 ± 0.007	0.190 ± 0.017
Calcium	0.607 ± 0.038	0.787 ± 0.123	1.77 ± 0.113	2.12 ± 0.084
Chloride	1.16 ± 0.130	1.83 ± 0.478	2.64 ± 0.666	2.95 ± 0.604
Sulphur	0.280 ± 0.008	0.313 ± 0.014	0.400 ± 0.052	0.367 ± 0.012
Potassium	2.60 ± 0.309	2.67 ± 0.562	3.20 ± 0.340	3.03 ± 0.223
Phosphorus	0.420 ± 0.028	0.343 ± 0.019	0.400 ± 0.021	0.367 ± 0.020
DCAD	464 ± 60.0	370 ± 16.5	297 ± 101.8	192 ± 140.2
K/Na Ratio	4.33 ± 1.186	3.00 ± 0.943	3.00 ± 0.471	2.67 ± 0.272
Catalpol			0.033 ± 0.008	0.032 ± 0.008
Aucubin			2.51 ± 1.01	1.88 ± 0.355
Acteoside			7.60 ± 2.05	4.93 ± 1.89

greater in plantain than pasture for both experiments. Of the secondary plant compounds measured, acteoside had the highest concentration in plantain (Table 2). Catalpol was present in very low concentrations (<0.09%). The concentration of all secondary compounds in plantain tended to be higher in the late lactation experiment.

Milk production and composition

Milk volume (L) and milk solids yield was greatest ($P < .05$) for pure plantain than ryegrass pasture in the late lactation experiment though there were no treatment differences in the early lactation experiment (Table 4). In both experiments, ryegrass and plantain (50–50) altered milk composition. In the late lactation experiment, milk fat percent was lower and lactose percent was higher when cows grazed 50–50 pasture–plantain compared with ryegrass pasture alone (Table 4). In the early lactation experiment, milk protein percent was lower for the 50–50 treatment than the ryegrass pasture treatment. Milk urea N was lowest ($P < .005$) for plantain in both experiments.

Urine and faeces

The concentration of urea and N in urine was lowest ($P < .001$) for plantain, intermediate for 50–50 pasture–plantain and highest for pasture in both experiments (Table 5). In the late lactation experiment, the concentration of NH_3 in the urine was lowest for plantain. There was no difference in NH_3 in the urine among treatments in the early lactation

Table 4. Mean milk yield and composition ($n = 12$) of dairy cows grazing perennial ryegrass-white clover pasture, plantain or 50–50 pasture–plantain.

	Pasture	50–50 pasture–plantain	Plantain	LSD	*P* value
Late lactation 2015					
Milk volume (L)	14.3^{a}	16.3^{b}	16.4^{b}	0.76	<.001
Milk solids (kg/day)	1.50^{a}	1.60^{ab}	1.67^{b}	0.08	.012
Milk protein (%)	4.28	4.29	4.34	0.09	.512
Milk protein (kg)	0.62^{a}	0.70^{b}	0.72^{b}	0.03	<.001
Milk fat (%)	6.16^{a}	5.52^{b}	5.80^{ab}	0.23	<.001
Milk fat (kg)	0.88	0.90	0.95	0.05	.142
Lactose (%)	4.95^{b}	5.07^{a}	5.05^{a}	0.04	<.001
Urea N (mmol/L)	11.2^{a}	10.9^{a}	9.96^{b}	0.54	.005
Early lactation 2015					
Milk volume (L)	27.1	27.9	26.9	1.14	.169
Milk solids (kg/day)	2.42	2.43	2.39	0.010	.772
Milk protein (%)	3.75^{a}	3.67^{b}	3.72^{ab}	0.060	<.001
Milk protein (kg)	1.02	1.03	1.00	0.037	.596
Milk fat (%)	5.48	5.32	5.38	0.230	.394
Milk fat (kg)	1.40	1.41	1.39	0.075	.886
Lactose (%)	5.18	5.15	5.16	0.043	.461
Urea N (mmol/L)	8.23^{a}	6.07^{b}	5.15^{c}	0.602	.001

Notes: LSD = least significant difference ($\alpha = 0.05$). Means within rows followed by different letters denote that values are significantly different at the 5% level.

experiment. Estimated urinary N output was lowest for plantain for all methods used in both experiments (Table 5).

In the late lactation experiment, there was no difference in the average urination frequency or volume between treatment groups as measured by the urine harness (Table 5). Using measured total urine volume per cow per day, daily N output was calculated to be 30% lower for cows on plantain (177 g N/cow/day) than those grazing pasture (251 g N/cow/day) in the late lactation experiment. In the early lactation experiment, daily N output was calculated to be over 40% lower for cows on plantain (119 g N/cow/day) and 50–50 pasture–plantain (114 g N/cow/day) than those on pasture (205 g N/cow/day)

The concentration of N in the faeces was similar among treatments in the late lactation experiment.

Microbial protein supply

Urinary PD concentrations and calculations associated with estimates of microbial N supply are presented in Table 6. The concentration of PD's allantoin, uric acid and hippuric acid was lowest ($P < .001$) for plantain in both experiments. All PD concentrations tended to be higher in the early lactation experiment than in the late lactation experiment for all treatments.

Discussion

The hypothesis of the present study was that the use of plantain as a grazed pasture species would be suitable to achieve similar milk production to that achieved by feeding standard ryegrass-white clover, while reducing urinary N losses. The results from this study support this hypothesis. In these experiments, pure plantain swards as 100% or 50% of a cow's

Table 5. Mean urine and faecal N characteristics ($n = 12$) and urine volume and urination frequency of dairy cows grazing perennial ryegrass-white clover pasture, plantain or 50–50 pasture–plantain.

	Pasture	50–50 pasture–plantain	Plantain	LSD	*P* value
Late lactation 2015					
Urine					
NH_3 (mmol/L)	1.98^{a}	1.35^{b}	0.97^{c}	0.30	<.001
Urea (mmol/L)	144.3^{a}	94.8^{b}	61.5^{c}	15.8	<.001
N concentration (g N/L)	5.4^{a}	3.6^{b}	2.4^{c}	0.06	<.001
Creatinine (mmol/L)	1.67^{a}	1.27^{b}	0.88^{c}	0.24	<.001
Total volume/day (L/day)[1]	46.5 (3)	59.1 (3)	73.8 (3)	23.65	.079
Urination frequency (events/day)[1]	18.0 (3)	18.3 (3)	20.0 (3)	6.66	.745
Average volume/urination (L)[2]	3.23 (95)	2.87 (88)	3.34 (86)	0.552	.228
N output using measured volume (g N/cow/day)	251	213	177		
N output using creatinine (g N/cow/day)	337^{a}	304^{b}	275^{b}	32.1	<.001
N output using MUN (g N/cow/day)	404^{a}	375^{b}	346^{c}	25.8	<.001
Faeces					
N (%)	3.43	3.33	3.45	0.11	.070
DM (%)	10.9^{a}	12.6^{b}	15.7^{c}	0.86	<.001
Early lactation 2015					
Urine					
NH_3 (mmol/L)	1.17	1.30	1.69	0.619	.239
Urea (mmol/L)	113.8^{a}	71.8^{b}	44.5^{c}	13.33	<.001
N concentration (g N/L)	4.7^{a}	3.4^{b}	2.2^{c}	0.046	<.001
Creatinine (mmol/L)	1.66^{a}	1.49^{ab}	1.26^{b}	0.238	.004
pH	8.09^{a}	7.92^{b}	7.67^{c}	0.127	<.001
Total volume/day (L/day)[1]	43.6 (2)	33.6 (2)	54.1 (3)	33.31	.331
Urination frequency (events/day)[1]	15.5 (2)	11.5 (2)	18.3 (3)	7.59	.141
Average volume/urination (L)[2]	2.75 (67)	2.82 (51)	3.09 (66)	0.491	.325
N output using measured volume (g N/cow/day)	205	114	119		
N output using creatinine (g N/cow/day)	298^{a}	232^{b}	184^{c}	24.19	<.001
N output using MUN (g N/cow/day)	304^{a}	218^{b}	189^{c}	17.7	<.001
Faeces					
N (%)	3.97^{a}	3.85^{a}	3.60^{b}	0.098	<.001
DM (%)	11.6	9.90	11.0	3.306	.581

Notes: LSD = least significant difference ($\alpha = 0.05$). Means within rows followed by different letters denote that values are significantly different at the 5% level.

[1] Values in parenthesis are the total number of cows on which urine meter data were recorded.

[2] Values in parenthesis represent the total number of measurements from the urine meter.

forage allowance reduced the concentration of N in the urine and estimated N excretion per day while maintaining or improving milk production.

Milk production

Milk solids produced per cow in late lactation was greatest for cows grazing plantain (1.67 kg/day) compared to pasture (1.50 kg/day) with 50–50 pasture–plantain intermediate (1.60 kg/day). This was largely due to an increase in milk volume as milk protein percentage was unaffected by pasture treatment and fat percentage tended to be lower where plantain was included in the diet. An overall increase in milk solids and a reduction in milk fat were also observed by Totty et al. (2013) when plantain was included in a mixed pasture. A possible explanation for the increase in milk volume was the observed increase in apparent dry matter intake (DMI) for cows on plantain in the late lactation experiment. This occurred because plantain was grazed to a lower post-grazing herbage mass compared with pasture despite similar daily allocations of herbage. However, it is noteworthy

Table 6. Mean urine purine derivative (PD) concentrations (mmol/L) of dairy cows ($n = 12$) grazing perennial ryegrass-white clover pasture, plantain or 50–50 pasture–plantain.

	Pasture	50–50 pasture–plantain	Plantain	LSD	*P* value
Late lactation 2015					
Allantoin	5.78^{a}	4.91^{b}	3.05^{c}	0.836	<.001
Uric acid	0.410^{a}	0.364^{a}	0.264^{b}	0.068	<.001
Hippuric acid	7.19^{a}	4.37^{b}	2.58^{c}	1.212	<.001
Xanthine	0.002	0.005	0.002	0.063	.386
Hypoxanthine	0.001^{a}	0.039^{b}	0.000^{a}	0.002	.006
PD index[1]	409^{b}	455^{a}	401^{b}	37.0	.009
Early lactation 2015					
Allantoin	9.71^{a}	8.81^{a}	6.63^{b}	1.205	<.001
Uric acid	0.769^{a}	0.695^{a}	0.490^{b}	0.1052	<.001
Hippuric acid	13.9^{a}	11.1^{b}	5.6^{c}	2.13	<.001
Xanthine	0.000	0.001	0.001	0.0010	.097
Hypoxanthine	0.001^{a}	0.003^{b}	0.003^{b}	0.0017	.034
PD index	720^{a}	682^{a}	617^{b}	54.5	<.001

Notes: LSD = least significant difference ($\alpha = 0.05$). Means within rows followed by different letters denote that values are significantly different at the 5% level.

[1] Purine derivative index (PD index) = $\{[\text{total PD (mmol/L)}]/\ \text{creatinine (mmol/L)}\} \times BW^{0.75}$.

that cows grazing 50–50 pasture–plantain had the highest apparent DMI in late lactation and this did not translate to increased milk production. In the early lactation experiment, milk production was not different between pasture types, despite some differences in apparent DMI. In a related study, Edwards et al. (2015) reported differences in DMI between simple and diverse pastures (15.3 ± 2.1 vs. 16.2 ± 1.4kg DM/cow/day, respectively) that did not change the milk yield and composition.

N partitioning

Previous short duration experiments have shown that when plantain is included in mixtures with ryegrass, white clover and chicory, urinary N concentration of dairy cows was reduced by at least 20% compared with perennial ryegrass-white clover pastures (Woodward et al. 2012; Totty et al. 2013). In the current experiment, when plantain was included in the diet at 50% or 100% of the allocation, urinary N concentration was more than 30% lower, in both experiments, compared with perennial ryegrass-white clover pasture only. The MUN concentration, an indicator of surplus dietary N (Cosgrove et al. 2014), was also lower for plantain-fed cows than for pasture-fed cows. Previous studies have shown the excretion of N in urine to be linearly related to N intake (Tas et al. 2006; Higgs et al. 2012). The current experiment indicated that factors other than N intake may have been a driver of lower urinary N concentration as there is no clear relationship with N intake across the dietary treatments. There is some evidence from *in vitro* studies that aucubin and acteoside may reduce rumen ammonia formation (Navarrete et al. 2016). There was no difference in faecal nitrogen, suggesting that this was not a route of increased excretion. Similar results have been seen by Judson and Edwards (2016) where urinary N concentration was reduced by about 30% for heifers supplemented with plantain silage despite having similar N intakes to those with no plantain in their diet. There may have been some differences in energy and protein supply to the rumen indicated by the lower PD index for cows on plantain (Chen 1989).

The urinary N concentration can be influenced by total urine volume. The total volume of urine excreted per day by animals grazing plantain (74 L) in the late lactation experiment measured by the urine harness was 57% greater than the volume of urine excreted by cows grazing pasture (47 L). Further, creatinine, a marker of urine volume (Chizzotti et al. 2008; Waldrip et al. 2013), was lower for cows on plantain diets in both experiments, which suggested a higher urinary output in both experiments for diets containing plantain. This may have been a factor of higher mineral content, secondary plant compounds or increased water intake due to the lower DM% of plantain. Plantain in both the late and early lactation experiments had a greater calcium and sodium mineral content than pasture. The concentration of sodium, a known diuretic (Ledgard et al. 2015), was almost twice as much in plantain compared with pasture but only in the late lactation experiment. This coincided with an observed increase in urine volume indicating that sodium may have been a factor. This increase in urine volume was supported by recent data, which strongly suggest a sustained diuretic effect when plantain was fed to sheep (O'Connell et al. 2016). Although the authors did not measure any secondary compounds, O'Connell et al. (2016) suggested that diuresis may have been due to bioactive compounds which are known to exist in the leaves of plantain.

Bioactive compounds in plantain have been studied in early medicinal work (Nishibi and Mural 1995). The major compounds in plantain include iridoid glucosides catalpol and aucubin and phenylethanoid glucoside acteoside. Although catalpol is a known diuretic (Tamura and Nishibe 2002), the availability of the compound in this experiment is unlikely to explain the higher urine volume output for cows grazing plantain as its concentration was low (<0.08 g/kg DM). The aucubin and acteoside were within range of findings from Navarrete et al. (2016) (aucubin 1.78–3.80 g/kg DM and acteoside 0.5–41.7 g/kg DM). These compounds may help to explain the greater urine output seen for animals grazing plantain pastures. The concentration of aucubin and acteoside was greatest in late lactation, which coincided with higher urine outputs. Aucubin is known to stimulate the excretion of uric acid from the kidneys (Kato 1946). However, in the current experiment, the concentration of uric acid in the urine was lower for cows grazing pure plantain compared with those on pasture. It is unclear why we observed this result and further research may be necessary.

Despite greater total volume output, N output was not increased for cows grazing plantain. The lower urinary N concentration from cows grazing plantain was able to offset the increase in calculated or measured urine volume, resulting in a reduction in total N output when plantain was included in the diet. Using average urination size and assuming a patch size of 0.2 m^2, the urine N loading from cows on perennial ryegrass-white clover pasture was approximately 700 kg N/ha in late lactation and 670 kg N/ha in early lactation. With the same assumptions, a urine patch from cows grazing plantain would have an N loading of approximately 450 kg N/ha in late lactation and 320 kg N/ha in early lactation. Application rates above 500 kg N/ha will likely increase leaching and nitrous oxide emission potential (Ledgard et al. 2007; Groenigen et al. 2010). This shows the potential of plantain to reduce N losses from grazing dairy systems. However, further research which defines what proportion of plantain in the diet is required to achieve reductions in N leaching and how to incorporate plantain into a grazing system is required.

Conclusions

Our results demonstrate a role for the use of plantain as a mitigation strategy to reduce the environmental impact of dairy farming. By providing plantain as a monoculture or with perennial ryegrass-white clover pastures to cows, milk solids production was increased or maintained and urine N concentration was reduced. The decline in urine N concentration may decrease urine patch N loading, thus reducing the risk of nitrate leaching for dairy grazing systems.

Acknowledgements

The programme is a partnership between DairyNZ, AgResearch, Plant & Feed Research, Lincoln University, Foundation for Arable Research and Landcare Research.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was undertaken as part of the Forages to Reduced Nitrate Leaching programme with principal funding from the Ministry of Business Innovation and Employment.

References

Bryant JR, Hoogendoorn CJ, Snow VO. 2007. Simulation of mitigation strategies to reduce nitrogen leaching from grazed pasture. Proceedings of the New Zealand Grassland Association. 69:145–151.

Bryant RH, Miller LA, Greenwood SL, Edwards GR. 2017. Milk yield and nitrogen excretion of dairy cows grazing binaryand multispecies pastures. Grass and Forage Science. 1–12. doi:10.1111/gfs.12274.

Chen XB. 1989. Excretion of purine derivatives by sheep and cattle and its use for the estimation of absorbed microbial protein [unpublished thesis]. Aberdeen, Scotland: University of Aberdeen.

Chen XB, Susmel P, Stefanon B, Orskov ER. 1995. Protein metabolism and nutrition. Proceedings of the 7th International Symposium on Protein Metabolism and Nutrition; Vale de Santelem, Portugal. p. 325–329.

Chizzotti ML, Valadares Filho SC, Valadares RFD, Chizzotti FHM, Tedeschi LO. 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. Livestock Science. 113:218–225.

Cosgrove GP, Taylor PS, Lowe KA, Foote AG, Jonker A. 2014. Milk urea estimates of nitrogen excretion by dairy cows grazing forage species with contrasting chemical and morphological characteristics. Proceedings of the 5th Australasian Dairy Science Symposium; Melbourne. p. 249–251.

CSIRO. 2007. Nutrient requirements of domesticated ruminants. Melbourne: CSIRO Publishing.

Di HJ, Cameron KC. 2007. Nitrate leaching losses and pasture yields as affected by different rates of animal urine nitrogen returns and application of a nitrification inhibitor – a lysimeter study. Nutrient Cycling and Agroecosystems. 79:281–290.

Edwards GR, Bryant RH, Smith N, Hague H, Taylor S, Ferris A, Farrell L. 2015. Milk production and urination behaviour of dairy cows grazing diverse and simple pastures. Proceedings of the New Zealand Society of Animal Production. 75:79–83.

Groenigen JW, Velthof GL, Oenema O, Groenigen KJ, Kessel C. 2010. Towards an agronomic assessment of N_2O emissions: a case study for arable crops. European Journal of Soil Science. 61:909–913.

Haultain J, Wigley K, Lee JM. 2014. Rising plate meters and a capacitance probe estimate the biomass of chicory and plantain monocultures with similar accuracy as for ryegrass-based pasture. Proceedings of the New Zealand Grassland Association. 76:67–74.

Higgs RJ, Chase LE, van Amburgh ME. 2012. Development and evaluation of equations in the Cornell net Carbohydrate and Protein System to predict nitrogen excretion in lactating dairy cows. Journal of Dairy Science. 95:2004–2014.

Jonker JS, Kohn RA, Erdman RA. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. Journal of Dairy Science. 81:2681–2692.

Judson HG, Edwards GR. 2016. Urinary nitrogen concentration from dairy heifers grazing kale supplemented with either plantain or perennial ryegrass baleage in winter. Journal of New Zealand Grasslands. 78:99–102.

Kato Y. 1946. Mechanism of uric acid excretion stimulation by aucubin. Folia Pharmacologica Japonica. 42:37–40.

Ledgard SF, Welten B, Betteridge K. 2015. Salt as a mitigation option for decreasing nitrogen leaching losses from grazed pastures. Journal of the Science of Food and Agriculture. 95(15):3033–3040.

Ledgard SF, Welten BG, Menneer JC, Betteridge K, Crush JR, Barton MD. 2007. New nitrogen mitigation technologies for evaluation in the Lake Taupo catchment. Proceedings of the New Zealand Grassland Association. 69:117–121.

Navarrete S, Kemp PD, Pain SJ, Back PJ. 2016. Bioactive compounds, aucubin and acteoside, in plantain (*Plantago lanceolata* L.) and their effect on in vitro rumen fermentation. Animal Feed Science and Technology. 222:158–167.

Nishibi S, Mural M. 1995. Bioactive components of plantago herb. Foods and Food Ingredients Journal. 166:43–49.

O'Connell CA, Judson HG, Barrell GK. 2016. Sustained diuretic effect of plantain when ingested by sheep. Proceedings of the New Zealand Society of Animal Production. 76:14–17.

Pacheco D, Burke JL, Death AF. 2007. Comparison of models for estimation of urinary nitrogen excretion from dairy cows fed fresh forages. Proceedings of the 3rd Australasian Dairy Science Symposium: Meeting the Challenges for Pasture-Based Dairying; Melbourne. p. 417–422.

Ravera B, Bryant RH, Cameron KC, Di HJ, Edwards GR, Smith N. 2015. Use of a urine meter to detect variation in urination behaviour of dairy cows on winter crops. Proceedings of the New Zealand Society of Animal Production. 75:84–88.

Tamminga S. 1992. Nutrition management of dairy cows as a contribution of pollution control. Journal of Dairy Science. 75:345–357.

Tamura Y, Nishibe S. 2002. Changes in the concentration of bioactive compounds in plantain leaves. Journal of Agricultural and Food Chemistry. 50:2514–2518.

Tas BM, Taweel HZ, Smit HJ, Elgersma A, Dijkstra J, Tamminga S. 2006. Utilisation of N in perennial ryegrass cultivars by stall-fed lactating dairy cows. Livestock Science. 100:159–168.

Totty VK, Greenwood SL, Bryant RH, Edwards GR. 2013. Nitrogen partitioning and milk production of dairy cows grazing simple and diverse pastures. Journal of Dairy Science. 96:141–149.

Verbic J, Chen XB, Macleod NA, Orskov ER. 1990. Excretion of purine derivatives by ruminants. Journal of Agriculture Science. 114:243–248.

Waldrip HM, Todd RW, Cole NA. 2013. Prediction of nitrogen excretion by beef cattle: a meta-analysis. Journal of Animal Science. 91:4290–4302.

Woodward SL, Waghorn GC, Bryant MA, Benton A. 2012. Can diverse pasture mixtures reduce nitrogen losses? Proceeding of the 5th Australasian Dairy Science Symposium; Melbourne. p. 463–464.

Diurnal changes in the nutritive composition of four forage species at high and low N fertiliser

L.A. BOX, G.R. EDWARDS and R.H. BRYANT
Agriculture and Life Sciences Faculty, Lincoln University, Lincoln, New Zealand
lisa.box@lincolnuni.ac.nz

Abstract

Chicory and plantain have been suggested as alternative grazed forages to perennial ryegrass for New Zealand dairy systems. While diurnal changes in plant chemical composition have been described for ryegrass there is currently little information for herbs. This experiment aimed to compare the effect of nitrogen inputs (low and high) and harvesting time (am versus pm) on the chemical composition of four forages (ryegrass, plantain, chicory and white clover). The effect of harvest time was greater than N fertiliser inputs on chemical composition for all forages. Ryegrass showed the greatest increase in water soluble carbohydrate diurnally, at the expense of neutral detergent fibre and to a lesser extent crude protein. This suggests afternoon allocation of ryegrass may be beneficial to improve the nutritive value of pasture on offer; allocation timing is less important for white clover, chicory and plantain.

Keywords: chicory, clover, crude protein, plantain, ryegrass, water soluble carbohydrate

Introduction

The composition of nutrients available to grazing animals in ryegrass pastures varies widely due to seasonal and diurnal changes. A ryegrass-based pasture can have low crude protein (CP) and high fibre concentrations in summer, which may restrict feed intake so that animal nutrient requirements are often not met (Waghorn 2002). At other times of the year ryegrass-based pastures can have a high concentration and solubility of CP (Bryant *et al.* 2012a) resulting in a low utilisation efficiency and a large proportion of dietary N being excreted in the urine (Tamminga 1992). Urinary N losses are an environmental concern and mitigation strategies are sought to meet new regulations. A positive relationship between CP intake and urinary N losses has been well established (Tas *et al.* 2006). Management strategies or pasture species which improve the nutritive value of pasture and reduce the N content may be valuable to pastoral grazing systems. One potential mitigation strategy may be to allocate pasture in the afternoon to reduce the proportion of N in the pasture and thus the supply to the animal (Moorby *et al.* 2006; Bryant *et al.* 2014).

Ryegrass shows a diurnal change in water soluble carbohydrate (WSC) concentration increasing from morning to night (Miller *et al.* 2001). This is offset by a decline in CP and fibre. In a previous experiment when an elevated WSC concentration was achieved in high sugar ryegrass by harvesting in the afternoon, urinary N output was reduced by 28.7 g/cow/day compared with a non-selected ryegrass harvested in the morning (Miller *et al.* 2001). Another mitigation option may be to use alternative plant species. Pastures which include herbs (plantain and chicory) have been identified as a means of reducing urinary N concentration compared with a ryegrass-white clover pasture, while maintaining or improving milk production (Bryant *et al.* 2012b; Totty *et al.* 2013; Edwards *et al.* 2015; Box *et al.* 2016). However, the mechanisms contributing to this effect are unclear.

While diurnal changes in the chemical composition of ryegrass have been well studied, less information is available for alternative herb and legume species (e.g. plantain, chicory, red and white clover). The purpose of this study was to compare the effect of N inputs and harvest time on herbage nutritive value of conventional and alternative pasture forages.

Materials and methods

Experimental site and design

The experiment was conducted from 8 October 2014 to 18 May 2015 at the Lincoln University Research Dairy Farm, Canterbury New Zealand (43º64'S, 172º46'E). A split-split plot design was used with three replicates. Treatments were forage type (4 species), N rate (two levels) and harvest time (am and pm). The four forage types were; diploid perennial ryegrass, plantain, chicory, and white clover (Table 1). N fertiliser was applied following each defoliation as calcium ammonium nitrate (27 : 0 : 0 : 0; N : P : K : S), with the total annual N application rate split evenly throughout the year. Before the experiment began, the planned N fertiliser treatments were 200 and 500 kg N/ha/year. It was estimated that nine harvests for legumes and ten harvests for grasses and herbs would occur. However, due to best practice methods, legumes were harvested seven times and grasses and herbs harvested nine times. This resulted in lower levels of N applied than proposed and differences in rates of N fertiliser applied between legumes and grasses and herbs. The final two fertiliser rates were low N inputs of 180 kg N/ha/year for grasses and herbs and 156 kg N/ha/year for legumes or high N

ISSN 2463-2872 (Print) ISSN 2463-2880 (Online)

inputs of 450 kg N/ha/year for grasses and herbs and 389 kg N/ha/year for legumes.

Management

All species were sown as monocultures in March 2014 using a Flexiseeder 14 row plot drill (2.1 m width) following cultivation. Plots (6.3 m^2) were managed as described by Martin *et al.* (2017). All species were grown under the same climatic and edaphic conditions with soils and nutrient (excluding nitrogen) being non-limiting to growth. The site was irrigated with a travelling irrigator between October and March, with ~20-30 mm water applied per week. Plots were fertilised with 12.8 kg P/ha, 20 kg K/ha and 32.8 kg S/ha in March and October 2014. Lime was applied in October 2014 at a rate of 2 t/ha.

Plots were managed by mowing with a Walker MC GHS ride-on rotary lawnmower, with mower height set to 4 cm for all species under a cut and carry regime. The timing of harvests was determined by best practices for maximising herbage growth and persistence of the three different functional groups: grasses, legumes and herbs (Moot *et al.* 2003; Lee *et al.* 2011). Grasses and herbs were defoliated at 32, 26 and 30 day intervals in spring, summer and autumn, respectively. Legumes were harvested at 41, 35 and 41 day intervals in spring, summer and autumn, respectively. Due to low soil temperatures and slow growth rates, no plots were harvested in winter.

Table 1 Forage species sown and their scientific name, cultivar and sowing rate.

Forage	Scientific name	Cultivar	Sowing rate (kg/ha)
Perennial ryegrass	*Lolium perenne*	One50 AR37	20
Chicory	*Cichorium intybus*	Choice	8
Plantain	*Plantago lanceolata*	Tonic	10
White clover	*Trifolium repens*	Kopu II	5

Table 2 Mean herbage crude protein (CP) concentration (g/kg DM) of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn) in the morning (AM) or afternoon (PM) at low and high N fertiliser inputs.

N inputs	Harvest Time	Forage	Spring	Summer	Autumn
Low	AM	Ryegrass	158	124	182
		Chicory	167	180	217
		Plantain	157	142	198
		White Clover	257	260	294
	PM	Ryegrass	143	115	166
		Chicory	166	162	193
		Plantain	145	131	179
		White Clover	246	246	277
High	AM	Ryegrass	210	158	220
		Chicory	188	223	246
		Plantain	186	163	204
		White Clover	274	250	295
	PM	Ryegrass	175	155	205
		Chicory	189	195	221
		Plantain	164	152	191
		White Clover	239	245	288
	SEM		2.8	2.2	2.4
Probabilities		Forage	<0.001	<0.001	<0.001
		N rate	<0.001	<0.001	<0.001
		Forage*N rate	0.070	0.002	0.017
		Time	<0.001	<0.001	<0.001
		Forage*Time	0.005	0.022	0.120
		N rate*Time	0.012	0.832	0.220
		Forage*time*Nrate	0.145	0.290	0.700

Herbage measurements

At each harvest samples were taken from each plot at 0700 and 1600 hours. A 1 m strip was cut at grazing height (4 cm) using electric hand-shears. Each sample was frozen and freeze-dried before being ground through a 1 mm sieve using a M200 rotor mill (Retsch Inc. Newtown, Pennsylvania, USA). Ground samples were scanned by near infra-red reflectance spectrophotometer (NIRS, NIRSystems 5000, Foss, Maryland, USA) to determine crude protein (CP), water soluble carbohydrates (WSC), fibre (NDF) and digestibility (DOMD). NIRS calibrations were created using samples from this experiment and from Martin *et al.* (2017). However, when samples were outside the calibration spectrum, wet chemistry was conducted. Data for each plot were averaged seasonally. Harvests in spring occurred between 9 October 2014 and 30 October 2014. Summer harvests occurred between 24 November 2014 and 10 February 2015 and autumn harvests between 10 March 2014 and 18 May 2015.

Statistical analysis

Data were averaged for each treatment seasonally. Data were analysed by spilt-split plot ANOVA using Genstat (VSN International LTD. 2102) with forage type as the whole plot, N rate as the split-plot and time of day as the split-split plot. Differences were considered significant at P<0.05.

Results

Crude protein

The CP concentration of white clover (average 264 g/kg DM) was the highest of all species and was unaffected by N fertiliser. Increasing N fertiliser inputs increased (P<0.001) the CP concentration of ryegrass, chicory and plantain by an average of 29 g/kg DM across all seasons (Table 2). The increase in CP with increasing fertiliser inputs for ryegrass, chicory and plantain was lower in spring (+ 13 g CP/kg DM) than summer (+ 30 g CP/kg DM) or autumn (+ 26 g CP/kg DM). Herbs had greater (P<0.001) CP concentration (average 184 g/kg DM) than ryegrass (average 166 g/kg DM) in autumn

Table 3 Mean herbage water soluble carbohydrate (WSC) concentration (g/kg DM) of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn) in the morning (AM) or afternoon (PM) at low and high N fertiliser inputs.

N inputs	Harvest Time	Forage	Spring	Summer	Autumn
Low	AM	Ryegrass	230	274	161
		Chicory	190	152	143
		Plantain	210	179	164
		White Clover	194	132	156
	PM	Ryegrass	295	333	234
		Chicory	241	189	202
		Plantain	227	202	214
		White Clover	225	163	193
High	AM	Ryegrass	192	222	142
		Chicory	186	151	151
		Plantain	202	176	177
		White Clover	175	149	165
	PM	Ryegrass	255	259	189
		Chicory	228	204	204
		Plantain	227	208	203
		White Clover	225	166	181
	SEM		4.2	3.2	2.6
Probabilities		Forage	0.033	<0.001	0.013
		N rate	0.021	0.021	0.019
		Forage*N rate	0.138	0.001	0.003
		Time	<0.001	<0.001	<0.001
		Forage*Time	<0.001	0.002	0.004
		N rate*Time	0.727	0.524	0.005
		Forage*time*Nrate	0.418	0.020	0.688

and summer. Across all seasons the CP concentration of grasses and herbs was reduced on average by 16 g CP/kg DM by harvesting in the afternoon regardless of N fertiliser rate. Chicory tended to have a greater CP concentration than plantain and a greater concentration of CP at the higher N rate in summer.

Water soluble carbohydrates

Increasing N inputs reduced (P=0.030) the WSC concentration of grasses by 39 g/kg in spring, 30 g/kg DM summer and 63 g/kg in autumn (Table 3). There was no N input effect on WSC concentration for herbs or legumes. White clover, chicory and plantain tended to have a lower WSC concentration than ryegrass across all seasons and treatments. Across all seasons, there was an increase in the WSC concentration from morning to afternoon for all species which was not affected by N rate. The effect of time of day on WSC concentration was different for each species. Ryegrass showed the greatest increase in WSC concentration diurnally. In autumn and spring, the increase in WSC concentration from morning to afternoon was close to twice that as the increase for herbs and about three times that in white clover. In spring and summer the increase in WSC concentration from morning to afternoon was greater (P=0.038) for chicory than plantain.

Fibre

The average concentration of NDF was two times greater for ryegrass than plantain, chicory or white clover across all seasons regardless of N rate (Table 4). The effect of time of day and N fertiliser on fibre concentration was inconsistent across forage types and seasons. In autumn and spring the diurnal reduction in NDF concentration of ryegrass was the greatest (P<0.001) of all forage types (average -26 g NDF/kg DM). There was no time of day effect on the NDF concentration of plantain in autumn or spring. The effect of harvest time on NDF concentration in summer was similar (P=0.082) for all forages. There was no effect on N fertiliser on the NDF concentration for all forages in in autumn and summer. In spring there was a small (>10 g NDF/kg DM) but

Table 4 Mean herbage neutral detergent fibre (NDF) concentration (g/kg DM) of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn) in the morning (AM) or afternoon (PM) at low and high N fertiliser inputs.

N inputs	Harvest Time	Forage	Spring	Summer	Autumn
Low	AM	Ryegrass	424	466	455
		Chicory	183	231	186
		Plantain	248	302	237
		White Clover	222	270	231
	PM	Ryegrass	391	434	431
		Chicory	158	227	185
		Plantain	221	303	233
		White Clover	215	254	218
High	AM	Ryegrass	403	467	443
		Chicory	176	227	202
		Plantain	228	297	236
		White Clover	216	263	225
	PM	Ryegrass	401	442	426
		Chicory	144	225	186
		Plantain	198	291	240
		White Clover	212	259	223
	SEM		6.0	4.4	5.1
Probabilities		Forage	<0.001	<0.001	<0.001
		N rate	0.004	0.537	0.767
		Forage*N rate	0.207	0.608	0.065
		Time	<0.001	0.002	<0.001
		Forage*Time	0.082	0.008	0.007
		N rate*Time	0.205	0.486	0.419
		Forage*time*Nrate	0.083	0.761	0.060

significant (P=0.004) reduction in NDF with increasing N inputs for all forages.

Digestibility

There was no influence of N fertiliser rate on DOMD for all species across all seasons (Table 5). Across all seasons chicory and white clover had the highest (P=0.002) DOMD regardless of harvest time. There was no diurnal influence on the DOMD of chicory and plantain in spring. In autumn the diurnal increase in DOMD was greatest for ryegrass (+ 29 g/kg DM), intermediate for chicory (+ 24 g/kg DM) and lowest for plantain and white clover (+ 14 and 11 g/kg DM, respectively). The diurnal increase in DOMD was similar for chicory and plantain (+ 16 and 10 g/kg DM, respectively) in summer. This was lower than the diurnal increase in DOMD ryegrass (+ 29 g/kg DM) and white clover (+ 25 g/kg DM) in summer.

WSC:CP ratio

The ratio of WSC to CP for all pasture species had a large seasonal variation (Table 6). White clover consistently had the lowest WSC:CP which was typically less than half that of the WSC:CP of ryegrass. For all seasons and pasture species, the WSC:CP ratio was increased by harvesting in the afternoon. Across all seasons and management treatments ryegrass had the highest WSC:CP which only fell below 1.0 for samples harvested in the morning during autumn.

Discussion

The aim of this study was to investigate the effects of time of day and N fertiliser inputs on chemical composition of four different forage species. Nitrogen inputs had little effects on the chemical composition of the forages. Increasing N fertiliser inputs did not improve DOMD and WSC nor reduce NDF concentration of any of the forages. Unsurprisingly, and similar to previous research (Elgersma & Hassink 1997; Martin *et al.* 2017) there was an increase in CP for ryegrass, chicory and plantain when N fertiliser inputs were increased and no effect of N fertiliser on the CP of

Table 5 Mean herbage digestibility of organic matter (DOMD) concentration (g/kg DM) of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn) in the morning (AM) or afternoon (PM) at low and high N fertiliser inputs.

N inputs	Harvest Time	Forage	Spring	Summer	Autumn
Low	AM	Ryegrass	781	753	757
		Chicory	808	770	812
		Plantain	774	728	775
		White Clover	807	753	806
	PM	Ryegrass	808	785	790
		Chicory	807	783	833
		Plantain	773	733	795
		White Clover	822	787	820
High	AM	Ryegrass	787	742	758
		Chicory	809	775	812
		Plantain	792	740	783
		White Clover	816	761	814
	PM	Ryegrass	796	769	783
		Chicory	817	794	839
		Plantain	788	753	792
		White Clover	823	777	823
	SEM		2.0	4.4	5.1
Probabilities		Forage	0.002	0.002	<0.001
		N rate	0.11	0.574	0.161
		Forage*N rate	0.277	0.146	0.197
		Time	0.002	<0.001	<0.001
		Forage*Time	0.007	0.032	0.006
		N rate*Time	0.208	0.749	0.231
		Forage*time*Nrate	0.099	0.329	0.286

white clover. This suggests altering N fertiliser inputs is not a beneficial management practice to improve forage quality (DOMD and WSC). The effect of harvest time on WSC, NDF and DOMD of all forages was greater than the effect of N fertiliser.

Diurnal changes in chemical composition of ryegrass have been previously used to create an increase in the concentration of WSC at the expense of CP with a goal of increasing the utilisation efficiency of dietary N (Miller *et al.* 2001; Moorby *et al.* 2006). The reduction in both CP and NDF as WSC increases would be favourable to animals grazing pastures in New Zealand in most circumstances. Experiments which utilised diurnal changes in plant chemical composition have inconsistently shown reductions in urinary N concentrations (Miller *et al.* 2001; Bryant *et al.* 2014). In the current experiment, forage types showed differences in their concentration of WSC and their diurnal accumulation of WSC. While harvesting in the afternoon increased the WSC concentrations of all species, ryegrass had the greatest accumulation of sugars diurnally. This did not always translate to larger reductions in CP compared with the other forages. For ryegrass, chicory and plantain, the average diurnal change in CP was small (<16 g CP/kg DM), however, when combined with the diurnal increase in WSC the WSC:CP ratio was altered.

Edwards *et al.* (2007) suggested a WSC:CP ratio above *c.* 0.7 can lead to a direct reduction in the proportion of N intake excreted in urine. This occurred for grasses and herbs in spring and summer, however, the WSC:CP ratio of legumes was typically below 0.7 across all seasons. There was a strong effect of harvesting in the afternoon on the WSC:CP ratio in autumn which was consistent across all species although greatest for ryegrass. This higher WSC:CP ratio in the afternoon may increase microbial protein synthesis efficiency and nitrogen-use efficiency of dairy cows (Vibart *et al.* 2012), as herbage provides more rapidly fermentable carbohydrates (Hristov *et al.* 2005) and produces less ammonia in the rumen (Tamminga 1996). The larger diurnal accumulation of WSC for ryegrass suggests that

Table 6 Mean herbage WSC:CP ratio of forages cut between 9 October and 30 October 2014 (spring), 24 November 2014 and 10 February 2015 (summer) or 10 March and 18 May 2015 (autumn) in the morning (AM) or afternoon (PM) at low and high N fertiliser inputs.

N inputs	Harvest Time	Forage	Spring	Summer	Autumn
Low	AM	Ryegrass	1.5	2.3	0.9
		Chicory	1.2	0.9	0.7
		Plantain	1.3	1.3	0.8
		White Clover	0.8	0.5	0.5
	PM	Ryegrass	0.9	1.5	0.6
		Chicory	1.0	0.7	0.6
		Plantain	1.1	1.1	0.9
		White Clover	0.6	0.6	0.6
High	AM	Ryegrass	2.1	3.0	1.4
		Chicory	1.5	1.2	1.1
		Plantain	1.6	1.6	1.2
		White Clover	0.9	0.7	0.7
	PM	Ryegrass	1.5	1.8	0.9
		Chicory	1.2	1.1	0.9
		Plantain	1.4	1.4	1.1
		White Clover	0.9	0.7	0.6
	SEM		0.55	0.50	0.23
Probabilities		Forage	0.001	<0.001	<0.001
		N rate	<0.001	<0.001	<0.001
		Forage*N rate	0.006	<0.001	0.006
		Time	<0.001	<0.001	<0.001
		Forage*Time	<0.001	<0.001	0.002
		N rate*Time	0.907	0.004	0.006
		Forage*time*Nrate	0.247	0.003	0.532

afternoon allocation was more important for ryegrass than chicory or plantain.

Under summer irrigation, grasses grown in Canterbury may not meet the CP requirements of high producing cows. In summer ryegrass at the lower N rate had an average CP concentration of 119 g CP/kg DM which is below the CP requirement for a high producing dairy cow (CSIRO 2007). At this time, increasing N inputs from 180 kg N/ha/year to 450 kg N/ha/year increased the CP concentration of ryegrass, chicory and plantain by an average of 3.2 g CP/kg DM. Chicory and plantain at low N inputs in summer had a higher average CP concentration (153 g CP/kg DM) than ryegrass (119 g CP/kg DM), which is consistent with the results of previous studies (Sanderson *et al.* 2003; Belesky *et al.* 2004). Where N fertiliser use is limited herbs may provide a suitable alternative to meet CP requirements. Chicory had the highest CP concentration regardless of N fertiliser rate or harvest time. This is consistent with findings of Martin *et al.* (2017) who showed chicory had a greater N concentration than plantain or ryegrass across fertiliser inputs ranging from 0 to 450 kg N/ha/year. This may lead to higher N intakes from chicory per unit of DM consumed compared with ryegrass or plantain. Despite this increased CP concentration, grazing experiments which include chicory in the diet of dairy cows have consistently shown a reduction in urinary N concentration (Totty *et al.* 2013; Bryant *et al.* 2017; Minneé *et al.* 2017) which suggests factors other than CP intake may drive lower N excretion in livestock grazing pasture containing chicory.

Fibre content for all pasture species increased over the summer. This was particularly prevalent in the grass species. For all forage types the proportion of NDF could be reduced by harvesting in the afternoon but could not be altered by N fertiliser. The diurnal reduction in NDF (average 13 g/kg DM) was typically larger than the average reduction in CP (10 g/kg DM). This diurnal reduction in NDF was generally greatest for ryegrass. The reduction in NDF, combined with a reduction in CP offset the diurnal increase in WSC. This contrasts with the findings of Cosgrove *et al.* (2009), who reported that a diurnal increase in WSC in three ryegrass cultivars was largely offset by reduction in CP and to a lesser extent NDF. Despite the differences both experiments show the potential of increased WSC concentration to enhance the nutritional value of forages, in particular ryegrass. For chicory, plantain and white clover the proportion of NDF was always below the recommended 35% for adequate rumen function (CSIRO 2007). The lower NDF of chicory and plantain has been recorded before in dairy grazing experiments (Totty *et al.* 2013; Box *et al.* 2016) where milk production was similar or greater, indicating that the low NDF of herbs or mixed swards was not negatively affecting milk production.

Conclusions

The diurnal effect on chemical composition was greater than the effects of N fertiliser inputs. Ryegrass showed the greatest diurnal accumulation of WSC concentration. However, this did not always translate to the largest reduction in CP from morning to afternoon as NDF reduction were typically greater than reductions in CP. In autumn, there was a strong effect of harvesting forages in the afternoon on WSC:CP, particularly for ryegrass. Afternoon allocation of ryegrass may offer a strategy to enhance the nutritive value of forages. Allocation timing appears to be less important for chicory and plantain.

ACKNOWLEDGEMENTS

This research was undertaken as part of the Forages to Reduced Nitrate Leaching programme with principal funding from the New Zealand Ministry of Business, Innovation and Employment. The programme is a partnership between DairyNZ, AgResearch, Plant & Feed Research, Lincoln University, The Foundation for Arable Research and Landcare Research.

REFERENCES

Belesky, D.P.; Ruckle, J.M.; Clapham, W.M. 2004. Dry-matter production, allocation and nutritive value of forage chicory cultivars as a function of nitrogen. *Journal of Agronomy and Crop Science 190*: 100-110.

Box, L.A.; Edwards, G.R.; Bryant, R.H. 2016. Milk production and urinary nitrogen excretion of dairy cows grazing perennial ryegrass-white clover and pure plantain pastures. *Proceedings of the New Zealand Society of Animal Production 76*: 18-21.

Bryant, R.H.; Gregorini, P.; Edwards, G.R. 2012a. Effects of N fertilisation, leaf appearance and time of day on N fractionation and chemical composition of *Lolium perenne* cultivars in spring. *Animal Feed Science and Technology 173*: 210-219.

Bryant, R.H.; Miller, M.E.; Edwards, G.R. 2012b. Grazing behaviour of dairy cows on simple and diverse swards in summer and autumn. *Proceedings of the New Zealand Society of Animal Production 72*: 106-110.

Bryant, R.H.; Dalley, D.E.; Gibbs, J.; Edwards, G.R. 2014. Effect of grazing management on herbage protein concentration, milk production and nitrogen excretion of dairy cows in mid-lactation. *Grass and Forage Science 69*: 644-654.

Bryant, R.H.; Miller, L.A.; Greenwood, S.L.; Edwards, G.R. 2017. Milk yield and nitrogen excretion of dairy cows grazing binary and multispecies pastures. *Grass and Forage Science*: http://onlinelibrary.wiley.com/wol1/doi/10.1111/gfs.12274/full

Cosgrove, G.P.; Koolaard, J.; Luo, D.; Burke, J.L.; Pacheco, D. 2009. The composition of high sugar ryegrasses. *Proceedings of the New Zealand Grassland Association 71*: 187-193.

CSIRO (Ed.) 2007. Nutrient requirements of domesticated ruminants, Collingwood, Australia.

Edwards, G.R.; Bryant, R.H.; Smith, N.; Hague, H.; Taylor, S.; Ferris, A.; Farrell, L. 2015. Milk production and urination behaviour of dairy cows grazing diverse and simple pastures. *Proceedings of the New Zealand Society of Animal Production 75*: 79-83.

Edwards, G.R.; Parsons, A.J.; Rasmussen, S. 2007. High sugar ryegrasses for dairy systems. *Meeting the Challenges for Pasture-Based Dairying*: 307-334.

Elgersma, A.; Hassink, J. 1997. Effects of white clover (*Trifolium repens* L.) on plant and soil nitrogen and soil organic matter in mixtures with perennial ryegrass (*Lolium perenne* L.). *Plant and Soil 197*: 177-186.

Hristov, A.; Ropp, J.; Grandeen, K.; Abedi, S.; Etter, R.; Melgar, A.; Foley, A. 2005. Effect of carbohydrate source on ammonia utilization in lactating dairy cows. *Journal of Animal Science 83*: 408-421.

Lee, J.; Hedley, P.; Roche, J. 2011. Grazing management: guidelines for optimal pasture growth and quality. *DairyNZ Technical Series September*: 6-10.

Martin, K.M.; Edwards, G.R.; Bryant, R.H.; Hodge, M.; Moir, J.; Chapman, D.F.; Cameron, K.C. 2017. Herbage dry-matter yield and nitrogen concentration of grass, legume and herb species grown at different nitrogen-fertiliser rates under irrigation. *Animal Production Science*: *57*: 1283-1288. (doi:https://doi.org/10.1071/AN16455).

Miller, L.A.; Moorby, J.M.; Davies, D.R.; Humphreys, M.O.; Scollan, N.D.; MacRae, J.C.; Theodorou, M.K. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne L*): milk production from late-lactation dairy cows. *Grass and Forage Science 56*: 383-394.

Minneé, E.M.K.; Waghorn, G.C.; Lee, J.M.; Clark, C.E.F. 2017. Including chicory or plantain in a perennial ryegrass/white clover-based diet of dairy cattle in late lactation: Feed intake, milk production and rumen digestion. *Animal Feed Science and Technology 227*: 52-61.

Moorby, J.M.; Evans, R.T.; Scollan, N.D.; MacRae, J.C. 2006. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in dairy cows in early lactation. *Grass and Forage Science 61*: 52-59.

Moot, D.J.; Brown, H.E.; Teixeira, E.I.; Pollock, K.M. 2003. Crop growth and development affect seasonal priorities for lucerne management. *Grassland Research and Practice Series 11*: 201-208.

Sanderson, M.A.; Labreveux, M.; Hall, M.H.; Elwinger, G.F. 2003. Nutritive value of chicory and English plantain forage. *Crop Science 43*: 1797-1804.

Tamminga, S. 1992. Nutrition management of dairy cows as a contribution of pollution control. *Journal of Dairy Science 75*: 345-357.

Tamminga, S. 1996. A review on environmental impacts of nutritional strategies in ruminants. *Journal of Animal Science 74*: 3112-3124.

Tas, B.M.; Taweel, H.Z.; Smit, H.J.; Elgersma, A.; Dijkstra, J.; Tamminga, S. 2006. Utilisation of N in perennial ryegrass cultivars by stall-fed lactating dairy cows. *Livestock Science 100*: 159-168.

Totty, V.K.; Greenwood, S.L.; Bryant, R.H.; Edwards, G.R. 2013. Nitrogen partitioning and milk production of dairy cows grazing simple and diverse pastures. *Journal of Dairy Science 96*: 141-149.

Vibart, R.; Tavendale, M.; Burke, J.; Pacheco, D. 2012. *In vitro* fermentation of [15N-] ryegrass and ruminal digesta of sheep grazing a ryegrass-based pasture in the morning or in the afternoon. *Proceedings of the New Zealand Society of Animal Production 72*: 100-105.

Waghorn, G.C. 2002. Can forages match concentrate diets for dairy production? *New Zealand Society of Animal Production 62*: 261-266.

In sacco digestion kinetics of plantain and ryegrass-white clover harvested in the morning and afternoon

LA Box*, GR Edwards and RH Bryant

Faculty of Agriculture and Life Sciences, PO Box 85084, Lincoln University, Lincoln 7647, Christchurch

**Corresponding author.* Email: lisa.box@lincolnuni.ac.nz

Abstract

The objective of this experiment was to compare rumen degradation characteristics of ryegrass (*Lolium perenne*), white clover (*Trifolium repens*) or plantain (*Plantago lanceolata*) harvested either in the morning (0700) or afternoon (1600). Fresh herbage material from each treatment was weighed into duplicate nylon bags and incubated in four lactating Jersey × Friesian dairy cows with rumen fistula. Differences in nutritive value between forage types was greater than effect of harvest time as was the effect on rumen degradation characteristics. Plantain had faster ($P<0.001$) DM disappearance rates than perennial ryegrass-white clover (12.2 vs 8.80 %/hr). The soluble fraction was greater ($P=0.001$) for forages harvested in the afternoon (25.2% of DM) than those in the morning (15.4% of DM) regardless of forage type. Nitrogen degradation was almost complete (>95% of N) and similar for all treatments ($P>0.05$). An interaction for effective degradability revealed there was no effect of harvest time on DM and OM for plantain. However, perennial ryegrass-white clover harvested in the afternoon has a greater effective degradability of DM and OM than that harvested in the morning. This suggested there is a larger influence of harvest time on some degradation characteristics of perennial ryegrass-white clover compared with plantain.

Keywords: diurnal; herb; *Lolium perenne*; *Plantago lanceolata*; rumen degradation; *Trifolium repens*

Introduction

In New Zealand dairy systems alternative forage types to perennial ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) (RWC) pastures such as plantain (*Plantago lanceolate*) are increasing in popularity. Plantain is a herb species which can produce at least as much dry matter (DM) as traditional RWC pastures (Moorhead et al. 2009; Martin et al. 2017). There is also evidence of greater milk production by cows grazing plantain (Box et al. 2016; Pembleton et al. 2016). Pembleton et al. (2016) reported improved milk production by cattle grazing a mixed sward of RWC and plantain (at 50% herbage dry matter intake (DMI)) compared with those grazing RWC alone in early lactation. Box et al. (2016) found cows grazing pure plantain swards produced 11% more milk than those grazing RWC in late lactation. The increase in milk production was attributed to an increase in DMI despite similar allocations.

One of the mechanisms that controls herbage DM intake is rumen fill (Allen 2014). Forages such as RWC, with a relatively large high content, are more slowly digested compared with forages, such as plantain, which has a low fibre content (John et al. 1987; Burke et al. 2002; Chaves et al. 2006). Fibre concentration, DM content and digestibility of forages show diurnal fluctuations. For example, RWC and plantain harvested in the afternoon had a lower fibre content than did herbage harvested in the morning (Cosgrove et al. 2009; Box et al. 2017). Changes in fibre content are offset by changes in other variables such as sugars and/or crude protein (CP) which can also influence degradation rate via availability of substrates for microbes. Mechanisms which lower the fibre content of forages may be useful to increase the rate of degradation and in turn improve herbage DM intake.

The rate and extent of degradation of DM, organic matter (OM) and nitrogen (N) in the rumen has implications on animal production and nitrogen use efficiency. Adequate balance of OM relative to N available in the rumen has the potential to improve microbial protein synthesis and increase metabolisable protein supply to the ruminant (Tas et al. 2006). Therefore, alternative forages, such as plantain, or diurnal allocation strategies which improve the proportion of OM degraded relative to N, may be beneficial in improving nitrogen use efficiency in dairy cows. The objective of this research was to determine the DM, OM, and N digestion kinetics of plantain and RWC herbage harvested in the morning and afternoon.

Materials and methods

Experimental design

Rumen degradation characteristics were determined by nylon bag incubations of plantain and RWC herbage. The experiment was a 2 × 2 factorial design. There were two pasture treatments of either plantain or RWC which were harvested at two times of the day; 0700 or 1600 h. Fresh herbage material of each treatment was weighed into duplicate nylon bags and incubated in four rumen-cannulated multiparous Jersey × Friesian lactating dairy cows on a pasture diet of spatially separate RWC (50%) and plantain (50%). Each cow received one treatment per run, there were four runs, so all cows received each treatment. The experiment was carried out at the Lincoln University Research Dairy Farm with the approval of the Lincoln University Animal Ethics Committee (AEC 398) in April 2016.

Forage preparation

Herbage for rumen incubations was collected from existing paddocks sown with either plantain (cv. Tonic) or perennial ryegrass (cv. Arrow) and white clover (cv. Kopu II). Perennial ryegrass-white clover pastures were established in March 2014 and the plantain pastures in December 2014. Perennial ryegrass-white clover for incubation consisted of 89% perennial ryegrass and 11% white clover and plantain samples consisted of 100% plantain leaf. For each treatment, four bags of herbage of approximately 1 kg each were harvested to grazing height using hand shears. Harvested RWC herbage was cut to ~2 cm lengths and plantain to approximately 2 cm^2 to replicate mastication. Herbage of approximately 40 g dry weight was weighed into individual dacron bags (10 x 15 cm with 50µ pore size). Bags of each treatment were attached to a 1 m galvanised chain and frozen at -16°C until required. A total of 16 chains were prepared, to represent each treatment for four runs using the four cows. Duplicate and triplicate for the 48 h interval - bags were attached to the chain using plastic cable ties (total 13 bags per chain). Two subsamples of herbage for each treatment were taken. The first was oven dried at 65°C for 48 h to determine DM % and the second subsample was freeze dried and ground through a 1 mm sieve. Ground material was NIRS scanned to determine chemical composition calculated from forage samples in Martin et al. (2017).

Rumen incubations

Removal of *in sacco* bags commenced at 0630 h between 11 and 23 April 2016 using incubation intervals of 3, 6, 9, 12, 24, and 48 h, including wash samples at 0 h. Following each incubation, duplicate bags were plunged into ice water and rumen debris removed, then washedby hand until water ran clear, then dried at 65°C for 48 hours. Total DM was recorded for each bag and subsamples were analysed for OM (ashed at 500 °C) and N (Elementar Vario MAX) concentration.

Digestion kinetics

Rumen factorial disappearance rate of DM, N, and OM was calculated using a nonlinear model based on equation 1. Effective degradability of DM, N, and OM was calculated using equation 2 fitted to constant rumen passage rate of 0.06 %/h.

Equation 1. Factorial disappearance, where P= disappearance, a= soluble fraction, b= potentially degradable fraction, e= exponential, c= fractional degradation rate (%/hour), and t= time (hour) (Ørskov et al. 2009)

$$P = a \times b \times (1 - e^{-ct})$$

Equation 2. Effective degradation (ED), where P= disappearance, a= soluble fraction, b= potentially degradable fraction, c= fractional degradation rate (%/hour), and k= rumen passage rate (%/hour) (Valderrama et al. 2011)

$$ED = a + \frac{b \times c}{c + k}$$

Equation 3. Effective rumen degradable protein (ERDP), where P= disappearance, a= soluble fraction, b= potentially degradable fraction, c= fractional degradation rate (%/hour), k= rumen passage rate (%/hour),CP=crude protein (%) and =efficiency of degradable protein (0.8) (Valderrama et al. 2011)

$$ERDP = a + \alpha \frac{b \times c}{c + k} \times CP$$

Statistical analysis

A two-way ANOVA (GenStat 15.1) was used to analyse the chemical composition of forages and parameter estimates to determine effects of forage type, harvest time and there interaction. Forage type and harvest time were fixed terms and cows were replicates for the degradation parameters estimated from the non-linear fits.

Results

The chemical composition of plantain and RWC harvested in the morning and afternoon is shown in Table 1. The proportion of fibre (NDF) was 36% lower ($P<0.001$) in plantain than RWC. The effect of time of day on the fibre content of herbage was greater for RWC than plantain. The NDF concentration of pasture was reduced by 8% by harvesting in the afternoon. The diurnal increase in DOMD was twice as large ($P=0.047$) for RWC than for plantain (+5.7 g/kg DM vs. +3.0 g/kg DM). While the difference in DOMD between plantain and RWC harvested in the morning was significant ($P=0.013$), the difference was small (0.5%). The DM% of plantain was two thirds that of RWC and had a smaller diurnal increase.

DM digestion kinetics

The *in sacco* DM disappearance data are illustrated in Figure 1a. From hours 6 to 24 after incubation, DM disappearance was more rapid ($P<0.001$) for plantain than for RWC. DM disappearance tended to be greater for pasture harvested in the afternoon than morning until 24 hours after incubation. Estimated DM fractions and degradation parameters are summarised in Table 2. The percentage of DM released during hand washing of un-incubated samples (soluble DM; fraction A) was greater for samples cut in the afternoon regardless of forage type. The fractional DM degradation rate per hour was greater for plantain compared with RWC. There was no effect of harvest time on the fractional degradation rate of DM per hour.

OM digestion kinetics

There was little effect of pasture type or time of day on the *in sacco* disappearance of OM (Figure 1b). The soluble fraction of OM was greater for samples harvested in the afternoon and was not different between forage types (Table 2). There was no effect of forage type or time of harvest on fractions a, b or c or the potential degradability of OM. The effective degradability was greater for plantain (average 67.3%) than for RWC (61.2%). Ryegrass-white clover harvested in the afternoon had a greater effective degradability of 64.2% than did RWC harvested in the

Table 1 Forage dry matter (DM) content (%), chemical composition (% of DM) and predicted metabolisable enery (ME) (MJ ME/kg DM) determine by near infrared reflectance spectroscopy of plantain and perennial ryegrass-white clover harveted at 0700 (AM) or 1600 h (PM).

	Plantain		Ryegrass-white clover			Significance		
	AM	PM	AM	PM	SEM	Forage	Time	F*T
DM	8.5	11.6	12.7	19.1	1.04	<0.001	<0.001	<0.001
ADF	21.3	21.3	24.2	21.2	0.648	<0.001	<0.001	<0.001
WSC	14.9	20.2	8.4	13.2	2.11	<0.001	<0.001	0.623
DOMD	73.4	76.4	73.9	79.6	1.22	0.013	<0.001	0.047
ME	11.7	12.2	11.8	12.7	0.195	0.013	<0.001	0.047
NDF	26.5	25.7	42.6	39.2	3.74	<0.001	<0.001	0.012
OM	86.1	87.2	91.2	91.0	1.13	<0.0013	0.032	0.003
CP	27.5	24.6	25.3	27.1	0.587	0.596	0.058	<0.001

Table 2 Mean dry matter (DM), organic matter (OM) and nitrogen (N) degrdation characteristics of ryegrass-white clover and plantain harvested at 0700 (AM) or 1600 (PM) defined as solube fraction (a), potentially soluble fraction (b), fractional dissapearnce rate (c), potentially degradable (PD), effective degradability at 0.06 h (ED) and effective rumen degradable protein (ERDP).

	Plantain		Ryegrass-white clover			Significance		
%	AM	PM	AM	PM	SEM	Forage	Time	F*T
Dry matter								
a	16.4	23.7	14.4	26.6	0.019	0.886	0.010	0.425
b	74.8	68.7	77.0	64.5	0.022	0.795	0.032	0.409
c (/hour)	12.5	11.9	8.01	9.58	0.008	0.050	0.761	0.487
PD	91.3	92.4	91.4	91.2	0.995	0.827	0.859	0.785
ED	66.7	68.7	57.6	65.1	1.32	<0.001	0.003	0.047
Organic matter								
a	20.5	24.5	17.4	26.8	0.018	0.891	0.090	0.452
b	68.5	65.8	74.0	63.0	0.076	0.664	0.121	0.318
c (/hour)	10.3	10.1	7.66	9.36	0.009	0.439	0.726	0.662
PD	88.9	90.3	91.4	89.9	0.954	0.576	0.968	0.529
ED	66.5	68.0	58.2	64.2	1.35	<0.001	0.005	0.043
Nitrogen								
a	2.4	10.3	14.6	20.2	0.035	0.005	0.049	0.704
b	93.9	85.5	80.9	75.3	0.040	0.042	0.188	0.784
c (/hour)	8.84	8.56	7.09	8.27	0.006	0.324	0.656	0.470
PD	96.3	95.8	95.5	95.5	1.14	0.846	0.931	0.938
ED	58.0	60.0	57.8	61.8	1.23	0.608	0.086	0.530
ERDP	151	136	151	162	3.46	0.004	0.575	0.006

morning (58.2%). There was no time-of-harvest effect on plantain samples.

N digestion kinetics

From hours 6 to 24 after incubation, N loss was greater ($P<0.001$) for plantain than for RWC, and greater for pastures harvested in the afternoon than those harvested in the morning (Figure 1c). The soluble and potentially soluble fraction of N was greater ($P<0.05$) for plantain than RWC (Table 2). Forages harvested in the afternoon had a greater soluble fraction of N than those harvested in the morning. There was no effect of forage type or time of day on the fractional degradation rate, potential degradability or effective degradability of N.

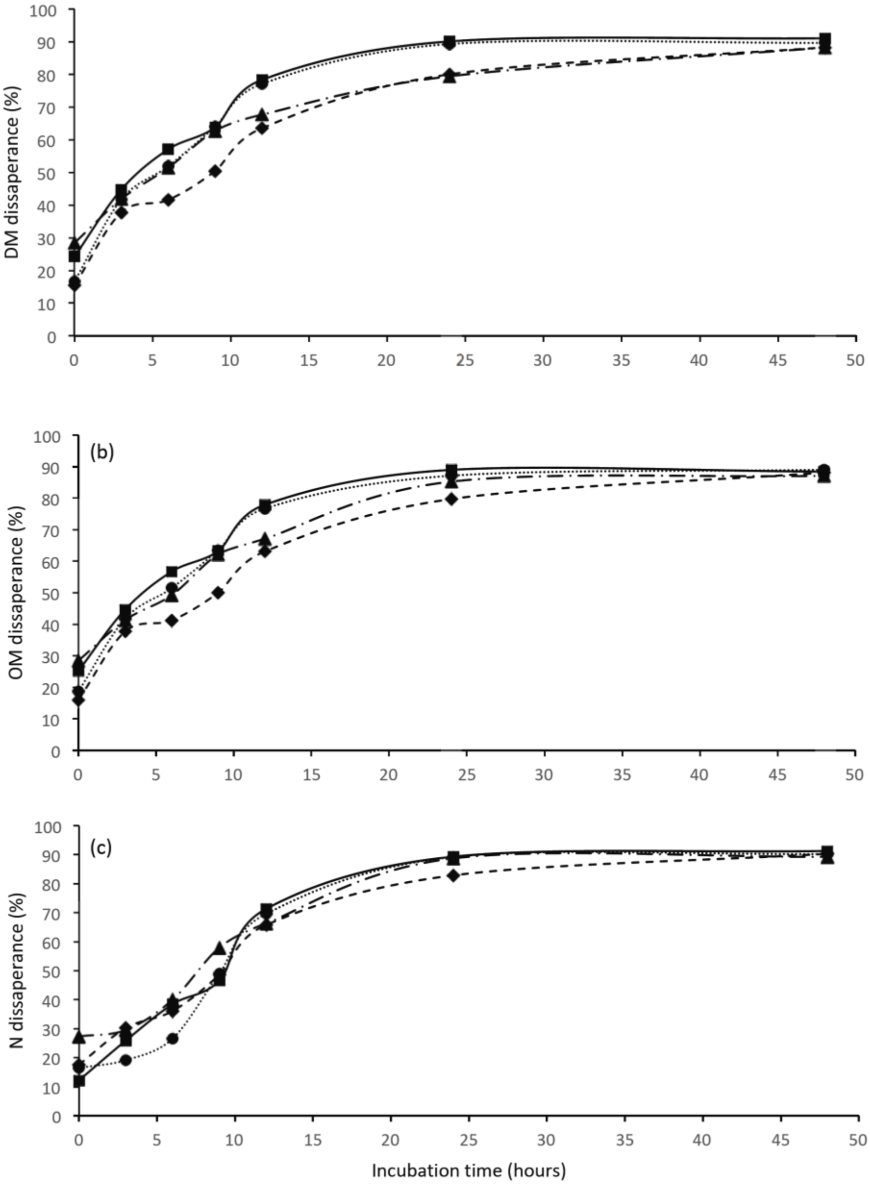
Discussion

This experiment compared the *in sacco* digestion kinetics of plantain and RWC harvested in the morning or afternoon to ascertain if digestion of forages was altered by management practises such as time of forage allocation. An interaction between forage and time revealed the effect of time of day on chemical composition and effective degradation of OM and DM was greater for RWC than for plantain. However, the effect of time of day on other degradation parameters was similar between forages.

Forage effect

In the current experiment, DM disappearance between 6 and 24 h, was more rapid for plantain than for RWC resulting in overall greater DM degradation rate. Although degradation rates in this experiment were much slower than those reported by Burke et al. (2000), the trend for plantain to have a faster rumen clearance time (25%/hr) compared with perennial ryegrass (11%/hr) was similar. Burke et al.

Figure 1 *In sacco* dissaperance of dry matter (DM) (a), organic matter (OM) (b) and nitrogen (N) (c) for ryegrass-white clover and plantain harvested at 0700 (AM) or 1600 (PM). Error bars are ±SEM. Plantain-AM(···•···), plantain-PM(—■—), RWC-AM (--◆---), RWC-PM (– ▲ –).



(2000) attributed the increased rumen clearance time of plantain to the structure of the plant cells and their rumen degradation characteristics. Plantain is known to contain significant concentrations of pectin, a structural but readily fermentable carbohydrate (Judson et al. 2009). These results support plantain as a mechanism to improve rumen clearance times via increased DM disappearance rates, which may be useful to increase herbage DM intake.

Cows grazing pastures which include plantain have consistently shown lower urinary N concentrations compared with those grazing RWC pastures (Box et al. 2016; Bryant et al. 2017; Minneé et al. 2017). Minneé et al. (2017) suggested the lower CP released from plantain in the early stages of *in sacco* degradation compared with perennial ryegrass may have contributed to lower urinary N concentration. In the current experiment the soluble fraction (A) of N was greater in RWC compared with plantain, which was similar to the results found by Minneé et al. (2017). However, there was no difference in the rate of degradation of N between RWC and plantain. Further, from 6 to 24 hours after incubation, the proportion of N which had disappeared from plantain was greater than that from RWC which was unexpected. Unlike Minneé et al. (2017) the N content of plantain and RWC was similar. Therefore, the greater N disappearance of plantain compared to RWC in this study was due to the increased rate of DM disappearance. For example, from hours 6, 9, 12 and 24, more DM had disappeared from plantain than from RWC, which coincided with the greater N loss.

Time-of-day effects

Forage type had a greater effect on the effective degradability and rate of degradation of DM and OM. However, the time of day that forages were harvested appeared to have a greater effect on the A and B digestion fractions of DM. Diurnally, there was an increase in the A fraction of DM for RWC and plantain, and a reduction in the B fraction. This meant that overall there was no time-of-day effect on the potentially degradable DM of RWC or plantain. This result was not surprising given there were only small increases in WSC of herbage and, therefore, small reductions in NDF. In this experiment the concentration of WSC was 5.3% DM higher for RWC and 4.9% DM higher for plantain harvested in the afternoon than for forages harvested in the morning. The magnitude of the difference was smaller than that reported by Cosgrove et al. (2007) of 6.1% DM for autumn harvested perennial ryegrass but similar to results found in a previous experiment at the same site. Box *et al*. (2017) found the diurnal increase in WSC for ryegrass was on average 5.8% DM greater for afternoon harvested perennial ryegrass and 2.9% DM greater for afternoon harvested plantain than forages harvested in the morning.

Despite no difference in the potential degradability of OM or DM between forage cut in the morning and afternoon, the effective degradability was improved when RWC and plantain were harvested in the afternoon. This diurnal effect was greater for RWC than plantain. This was likely due to RWC having larger changes in chemical composition diurnally. Notably the diurnal reduction in fibre was greater for RWC than that for plantain. This is similar to results found by Box et al. (2017), where there was a greater diurnal change in the nutritive value of perennial ryegrass, than plantain. Management strategies which allow allocation of RWC in the afternoon and plantain in the morning may provide benefits in milk production, through increased DM intake.

Conclusions

The results showed that different forages have different degradation parameters and these are influenced by diurnal effects on the relative chemical composition. Plantain degrades faster than RWC, which resulted in faster N degradation rates. As a result the use of plantain as an alternative forage for dairy systems may be useful to increase voluntary herbage DM intake. Effective degradability of DM and OM was improved when RWC and plantain were harvested in the afternoon. This diurnal effect was greater for RWC than for plantain.

References

Allen MS 2014. Drives and limits to feed intake in ruminants. Animal Production Science 54: 1513-1524.

Box LA, Edwards GR, Bryant RH 2016. Milk production and urinary nitrogen excretion of dairy cows grazing perennial ryegrass-white clover and pure plantain pastures. Proceedings of the New Zealand Society of Animal Production 76: 18-21.

Box LA, Edwards GR, Bryant RH 2017. Diurnal changes in the nutritive composition of four forage species at high and low N fertiliser. Journal of New Zealand Grasslands 79: 43-50.

Bryant RH, Miller LA, Greenwood SL, Edwards GR 2017. Milk yield and nitrogen excretion of dairy cows grazing binary and multispecies pastures. Grass and Forage Science 00: 1-12.

Burke JL, Waghorn GC, Chaves AV 2002. Improving animal performance using forage-based diets. Proceedings of the New Zealand Society of Animal Production 62: 267-272.

Burke JL, Waghorn GC, Brookes IM, Attwood GT, Kolver ES 2000. Formulating total mixed rations from forages - defining the digestion kinetics of contrasting species. Proceedings of the New Zealand Society of Animal Production 60: 9-14.

Chaves AV, Burke JL, Waghorn GC, Brookes IM 2006. Digestion kinetics of leaf, stem and inflorescence from five species of mature grasses. Journal of the Science of Food and Agriculture 86: 816-825.

Cosgrove GP, Koolaard J, Luo D, Burke JL, Pacheco D 2009. The composition of high sugar ryegrasses. Proceedings of the New Zealand Grassland Association 71: 187-193.

Cosgrove GP, Pacheco D, Burke JL, Death AF, Hickey MJ, Lane GA 2007. Effects of water soluble carbohydrate in forage on aspects of nitrogen partitioning in cows. In: Chapman DF, Clark DA, Macmillan KL, Nation DP ed. Meeting the challenges for pasture-based dairying. Proceedings of the 3rd Australian Dairy Science Symposium. Pp. 350-358.

John A, Ulyatt MJ 1987. Importance of dry matter content to voluntary intake of fresh grass forages. Proceedings of the New Zealand Society of Animal Production 47: 13-16.

Judson HG, McAnulty R, Sedcole R 2009. Evaluation of ‘Ceres Tonic’ plantain (*Plantago lancelota*) as a lactation feed for twin bearing ewes. Proceedings of the New Zealand Grassland Association 71: 201-205.

Martin KM, Edwards GR, Bryant RHet al. 2017. Herbage dry-matter yield and nitrogen concentration of grass, legume and herb species grown at different nitrogen-fertiliser rates under irrigation. Animal Production Science: -.

Minneé EMK, Waghorn GC, Lee JM, Clark CEF 2017. Including chicory or plantain in a perennial ryegrass/white clover-based diet of dairy cattle in late lactation: Feed intake, milk production and rumen digestion. Animal Feed Science and Technology 227: 52-61.

Moorhead AJE, Piggot GJ 2009. The performance of pasture mixes containing ‘Ceres Tonic’ plantain (Plantago lanceolata) in Northland. Proceedings of the New Zealand Grassland Association 71: 195-199.

Ørskov ER, McDonald I 2009. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. The Journal of Agricultural Science 92: 499-503.

Pembleton KG, Hills JL, Freeman MJ, McLaren DK, French M, Rawnsley RP 2016. More milk from forage: Milk production, blood metabolites, and forage intake of dairy cows grazing pasture mixtures and spatially adjacent monocultures. Journal of Dairy Science 99: 3512-3528.

Tas BM, Taweel HZ, Smit HJ, Elgersma A, Dijkstra J, Tamminga S 2006. Rumen degradation characteristics of perennial ryegrass cultivars during the growing season. Animal Feed Science and Technology 131: 103-120.

Valderrama X, Anrique R 2011. In situ rumen degradation kinetics of high-protein forage crops in temperate climates. Chilean Journal of Agricultural Research 71: 572-577.

NEW ZEALAND JOURNAL OF AGRICULTURAL RESEARCH
https://doi.org/10.1080/00288233.2018.1505641

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Seasonal and diurnal changes in aucubin, catalpol and acteoside concentration of plantain herbage grown at high and low N fertiliser inputs

Lisa A. Box, Grant R. Edwards and Racheal H. Bryant

Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln, New Zealand

ABSTRACT

This experiment aimed to characterise the concentration of secondary compounds aucubin, catalpol and acteoside in plantain (*Plantago lanceolata*) herbage and determine if harvesting at different times or nitrogen (N) fertiliser inputs alter concentrations. Herbage of plantain was harvested each season over two years from plots fertilised at 180 or 450 kg N/ha/yr. At each harvest date, samples were taken in at 0700 h and 1600 h. The concentration of secondary compounds in plantain herbage was greatest for acteoside (range 1.77-36.0 mg/g DM), intermediate for aucubin (range 0.566–5.85 mg/g DM) and lowest for catalpol (<0.390 mg/g DM). Compound concentrations were unaffected by N fertiliser and showed limited diurnal fluctuation. There was a strong seasonal effect on all secondary compounds with peaks in concentration occurring in spring for aucubin and acteoside. This experiment suggests environmental factors other than soil N fertility and diurnal patterns influence the concentration of secondary compounds in plantain.

Abbreviations: DR: digestibility-reducing; DM: dry matter; HPLC: High performance liquid chromatography; N: nitrogen; NH_3: ammonia

ARTICLE HISTORY
Received 9 March 2018
Accepted 25 July 2018

KEYWORDS
Bioactive; *Plantago lanceolata*; seasonal variation; secondary compounds; tonic

Introduction

Plantain (*Plantago lanceolata* L.) is a perennial herb which has been used in herbal medicine for human health and as a grazed forage species (Deaker et al. 1994; Stewart 1996; Box et al. 2017). Plantain can contain high levels of secondary plant compounds which are thought to have antimicrobial properties (Rumball et al. 1997; Navarrete et al. 2016) and anti-oxidative activity (Zhou and Zheng 1991; Darrow and Bowers 1999). Secondary plant compounds are those which are produced by plants that do not act towards primary growth or development of the plant, but can contribute to plant fitness through antimicrobial and allelopathic activity (Bourgaud et al. 2001). The potential role of plantain in environmental mitigation of grazing systems has placed an emphasis on mechanisms contributing to this effect including secondary plant compound presence (Gardiner et al. 2016; O'Connell et al. 2016;

CONTACT L.A. Box ✉ lisa.box@agresearch.co.nz

Box et al. 2017; Cheng et al. 2017). Plantain is known to have three main secondary plant compounds: aucubin, catalpol and acteoside (verbascoside) (Tamura and Nishibe 2002; Gardiner et al. 2016).

Aucubin and catalpol are iridoid glycosides which have shown anti-inflammatory properties in mice (Watkinson and Kear 1994; Pae et al. 2003). Furthermore, aucubin stimulates both the removal of uric acid from tissues to blood and the excretion of uric acid from the kidneys (Kato 1946). Acteoside is a phenylpropanoid glycoside that can be present in high levels (up to 100 mg/g DM) within the leaves of plantain (Fajer et al. 1992). Acteoside is known for its antimicrobial (Andary et al. 1982; Chen et al. 2012), and antioxidant effects (Zhou and Zheng 1991; Chen et al. 2012). Navarrete et al. (2016) showed these compounds may be useful in altering the partitioning of N in ruminants by acting as an energy source (when current levels are rate limiting) for microbes and reducing ammonia (NH_3) production and subsequent N losses in the urine (Box et al., 2017). These compounds show importance in livestock production systems, but to improve their effectiveness, an understanding of management strategies which accentuate concentrations of secondary plant compounds in plantain herbage.

According to Stewart (1996), plantain contains around 41 mg/g DM aucubin in the leaves. Catalpol is generally present at 7–50 mg/g DM (Tamura and Nishibe 2002). Acteoside can be found in differing concentrations depending on the time of year that plantain is harvested. Spring harvested plantain-contained concentrations of acteoside at 15 mg/g DM which increased to 41 mg/g DM in autumn (Tamura and Nishibe 2002). The concentration of secondary compounds in plantain herbage is influenced by genetic and environmental factors (Fajer et al. 1992; Tamura and Nishibe 2002). Differences in environmental factors such as soil nutrient availability, UV light and temperature have previously altered the secondary compound content of plantain (Fajer et al. 1992; Darrow and Bowers 1999; Tamura 2001). For example, when the Hoaglands nutrient solution was added to *Plantago* plants, the concentration of secondary compounds was greater than for those grown under no nutrient additions (Fajer. et al. 1992). Furthermore, the chemical composition of plantain has been altered by harvesting at different times of day and N fertiliser inputs, in an effort to reduce N losses from grazing systems (Box et al. 2016; Martin et al. 2017). This represents an opportunity to utilise harvesting at different times of day and N fertiliser rate, as management strategies to alter the secondary compound composition of plantain herbage. There is currently little information which quantifies the concentration of secondary compounds in plantain grown in temperate conditions such as occurs in New Zealand. This experiment characterised the seasonal change in the secondary plant compounds in the two growing seasons following establishment of Ceres Tonic plantain. This experiment also examined the effects of management such as nitrogen (N) fertiliser inputs and harvesting at different times of day on the concentration of secondary plant compounds.

Materials and methods

Experimental site and design

The experiment was conducted over two growing seasons from 29 October 2014 to 31 March 2016 at Lincoln, Canterbury New Zealand (43°64′ S, 172°46′ E) on a free-draining

Templeton fine sandy loam (Immature Pallic soil). A 2 × 2 × 3 factorial design was used over two years with three replicates of each treatment (area = 6.3 m^2). Treatments were N fertiliser rate (180 and 450 kg N/ha/year), harvest time (am (0700 h) and pm (1600 h)) and season (spring, summer and autumn). The experiment used plantain plots sown as a larger experiment (Martin et al. 2017), which had been established in March 2014 following cultivation. Plots were sown with plantain (cultivar, Ceres Tonic) at 10 kg/ha on 20 March 2014. Control of herbage mass commenced in spring 2014. Plots were harvest nine times per year from 8 October 2014 to 18 May 2015 in year 1 and 3 October 2014 to 3 May 2016 in year 2 using a ride-on mower, with cutting height set to 5 cm. Intervals between harvests were 32 days in spring, 26 days in summer and 30 days in autumn.

Soil nutrient sampling was conducted before the experiment with a soil corer to a depth of 75 mm; results showed pH = 6.1 (soil:water ratio, 1:2), Olsen phosphorus (P) = 26 mg/L (Olsen et al. 1954), sulfate-sulfur (sulfate-S) = 5 mg/kg (Watkinson and Kear 1994) and potassium (K) = 0.23 milliequivalents/100 g (Rayment and Higginson 1992). On the basis of this 12.8 kg P/ha, 20 kg K/ha and 32.8 kg S/ha was applied in March 2014 and October 2014 as well as 2 t lime/ha in October 2014.

Nitrogen fertiliser was applied as calcium ammonium nitrate (27:0:0:0; N:P:K:S), following each defoliation with the total annual N application rate split evenly throughout the year. Before the experiment began, the planned N fertiliser treatments were 200 and 500 kg N/ha/year. It was assumed there would be 10 harvests per year. However, following best management practices to maximise plantain yield, only nine harvests per year occurred. This resulted in fertiliser rates of 180 kg N/ha/year for the low N inputs treatment and 450 kg N/ha/year for high N input treatment. In year two of the experiment herbicide T Max (active ingredient aminopyralid) was applied at 40 mL/10 L water on the 7 October 2015 to remove dicotyledon species, particularly white clover. Herbicide Gallant (5 mL/10 L water) with surfactant Uptake (50 ml/20 L water) was applied on 4 December 2014, 22 January, 4 May and 10 June 2015 to remove grass species.

Meteorological data

Rainfall and air temperatures for the experimental period are presented in Figure 1. Total annual rainfall during the first year of experiment (386 mm) was lower than the long-term average rainfall of the last 30 years (599 mm) (Figure 1A). This shortfall in rain was compensated with irrigation, which was applied at a rate of 23 mm/week, between November 2014 and March 2015. In the second year rainfall varied, with around twice the long-term average occurring in February and June (Figure 1A). In spring and autumn of year 2, rainfall fell below the long-term average. This was compensated with irrigation which was applied at a rate of approximately 30 mm/week when rainfall was insufficient. The monthly air temperatures (Figure 1B) showed a similar trend to the long-term average air temperature.

Herbage measurements

Samples were collected from each plot at 0700 h and 1600 h once per season following a 25 day regrowth interval (Table 1). At each sampling date a 1 m strip was cut at to 4 cm

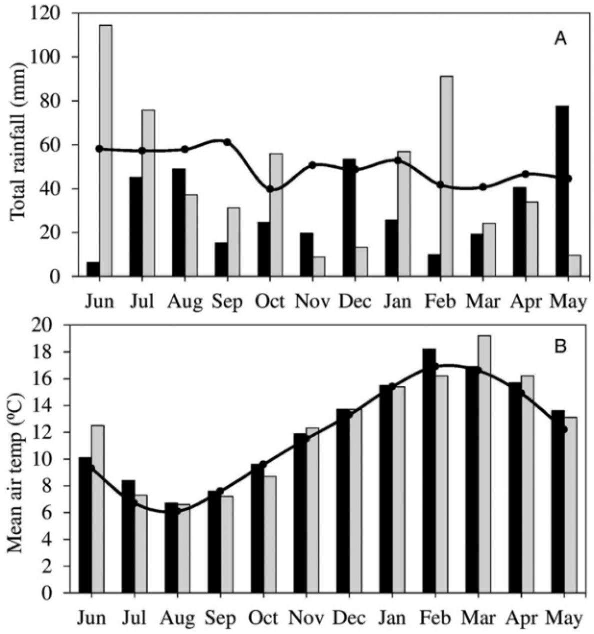


Figure 1. A, Monthly mean monthly rainfall. **B**, Mean air temperature for June 2014–May 2015 (year 1) and June 2015–May 206 (year 2) at Lincoln, Canterbury, New Zealand. Data were collected from Broadfields Meteorological Station, 1 km from research site. June 2014–May 2015 (■), June 2015–May 2016 (▨), long-term average (1981–2010) (——).

height using electric hand shears. To prevent degradation of the bioactive compounds in P. *lanceolata*, freeze-drying (Tamura and Nishibe 2002) and storage in a dark and cold place is necessary. Therefore, fresh herbage was put into sealed plastic bags, deep-frozen, and stored at −18°C until freeze-drying.

Freeze-dried samples were ground through a 1 mm sieve using a M200 rotor mill (Retsch Inc. Newtown, Pennsylvania, USA). Ground material was stored in a temperature controlled dark storage room then analysed for the secondary plant compounds catalpol, aucubin and acteoside using high performance liquid chromatography (HPLC) by Massey University analytical laboratory following procedures of Tamura and Nishibe (2002). A 100 mg aliquot from each of the ground samples was taken for extraction of aucubin, catalpol and acteoside, with 10 mL of methanol (MeOH) in 15 mL tubes and shaken for 2 h at

Table 1. Sampling dates of plantain herbage in year 1 (2014/15) and year 2 (2015/16) for HPLC analysis of secondary compounds.

Year	Season	Sampling date
1	Spring	29 October 2014
	Summer	13 January 2015
	Autumn	3 April 2015
2	Spring	6 October 2015
	Summer	2 February 2016
	Autumn	31 March 2016

room temperature. Solid plant material was removed (using grade 41 quantitative filter papers). For catalpol and aucubin, 2 mL of the filtrate was diluted in 8 mL of ultra-pure water, and further filtered using 0.2 µm syringe filters. A 20 µL aliquot of the diluted filtrate was used for simultaneous HPLC analysis of catalpol and aucubin. For acteoside, the undiluted filtrate further filtered using 0.2 µm syringe filter and a 20 µL of aliquot used for HPLC analysis.

Commercially available catalpol, aucubin and acteoside (99% pure) were used as standards. The standard solution contained 2 mg each of catalpol and aucubin in 50 mL of 20% MeOH and 1 mg of acteoside in 5 mL of pure MeOH. The mobile phase was 1% acetronitrile in water for catalpol and aucubin and 29% MeOH in water (containing 5% acetic acid) for acteoside. The flow rate was 1 mL/min. For catalpol and aucubin, wavelength detection was performed at 204 nm and for acteoside at 330 nm. HPLC was performed at 40°C.

Statistical analysis

Data were analysed for variance using the general linear model in GenStat (VSN International LTD. 2102) with replicates as random effect and year, season, N rate and time of day as fixed effects. Differences were considered significant at $P < .05$.

Results

Aucubin

All statistical outputs are shown in Table 2. An effect of year showed greater aucubin concentration in year 1 (2.56 mg/g DM) than year 2 (1.86 mg/g DM) (Table 3). An interaction between year and season showed that plantain had a greater concentration of aucubin in spring of the year 1 (average 2.18 mg/g DM) than year 2 (average 1.89 mg/g DM). In autumn, there was no difference ($P > .050$) in the average aucubin concentration between year 1 and 2. Aucubin concentration was lowest in summer. In both years the lowest concentration of aucubin occurred during the summer compared with other

Table 2. Probability (P) values showing the effects of nitrogen (N) fertiliser rate, time of day (time), year and season and their interaction on the concentration of acteoside, aucubin and catalpol in plantain herbage.

Factor	Acetoside	Aucubin	Catalpol
N rate	0.005	0.797	0.638
Time	0.001	0.243	0.424
Year	0.102	0.001	<0.001
Season	<0.001	<0.001	<0.001
N rate × time	0.059	0.300	0.237
N rate × year	0.054	0.368	0.679
Time × year	0.056	0.100	0.937
N rate × season	0.026	0.286	0.012
Time × season	<0.001	0.022	0.060
Year × season	0.378	<0.001	<0.001
N rate × time × year	0.145	0.277	0.389
N rate × time × season	0.251	0.966	0.001
N rate × year × season	0.072	0.094	0.002
Time × year × season	0.962	0.323	0.105
N rate × time × year × season	0.420	0.527	<0.001

Table 3. Mean herbage aucubin concentration (mg/g DM) (±SEM) of plantain harvested in year 1 (2014/15) or year 2 (2015/16) after establishment at 450 kg N/ha/yr and 180 kg N/ha/yr fertiliser inputs harvested either in the morning (AM) or afternoon (PM).

Year	*N* fertiliser (kg/ha/y)	Time	Spring	Summer	Autumn
1	180	AM	5.85 ± 0.602	1.28 ± 0.116	2.42 ± 0.801
		PM	3.86 ± 0.250	0.899 ± 0.065	1.73 ± 0.043
	450	AM	3.93 ± 0.377	1.07 ± 0.220	2.52 ± 0.387
		PM	3.20 ± 0.297	1.72 ± 0.452	2.23 ± 0.088
2	180	AM	2.13 ± 0.163	0.708 ± 0.144	2.34 ± 0.224
		PM	1.66 ± 0.406	0.922 ± 0.147	2.97 ± 0.565
	450	AM	2.77 ± 0.159	0.574 ± 0.240	2.31 ± 0.986
		PM	1.68 ± 0.579	0.566 ± 0.123	3.68 ± 0.208

seasons. The concentration of aucubin in plantain was not affected by N fertiliser rate or time of day.

Acteoside

The effect of treatments on the concentration of acteoside is shown in Table 2. The concentration of acteoside was highest in spring than other seasons. An interaction of season with time showed that acteoside concentration was unaffected by time of day in autumn and summer but was lower in the afternoon than morning in spring (Table 4). This diurnal effect was greater ($P < .050$) for plantain fertilised with 180 kg N/ha/yr. Nitrogen fertiliser rate of 180 kg N/ha/yr resulted in a greater acteoside concentration, though an interaction with year showed the effect of fertiliser was more pronounced in year 1 than year 2. Moreover, the interaction between N rate and season showed the variation in N response occurred in spring.

Catalpol

Catalpol concentration was consistently low at less than 1.0 mg/kg DM (Table 4). The concentration of catalpol was considerably higher in year 1 than year 2, with the concentration in year 2 falling below 0.05 mg/g DM for all treatments. An interaction between all factors revealed that in year 1, N fertiliser, season and time of day influenced catalpol concentration. For instance, at 180 kg N/ha/yr catalpol was highest in spring and summer compared with autumn. During summer, catalpol concentration was lower in the afternoon than the morning for the 180 kg N/ha/yr treatment but the reverse was true of 450 kg N/ha/yr treatment at the same time.

Table 4. Mean herbage acteoside concentration (mg/g DM) (±SEM) of plantain harvested in year 1 (2014/15) or year 2 (2015/16) after establishment at 450 kg N/ha/yr and 180 kg N/ha/yr fertiliser inputs harvested either in the morning (AM) or afternoon (PM).

Year	*N* fertiliser (kg/ha/y)	Time	Spring	Summer	Autumn
1	180	AM	36.0 ± 3.83	5.86 ± 1.70	10.2 ± 4.33
		PM	14.5 ± 0.266	1.77 ± 0.291	8.86 ± 1.80
	450	AM	16.7 ± 1.32	2.32 ± 0.115	8.08 ± 0.612
		PM	9.08 ± 1.92	4.53 ± 1.58	6.49 ± 0.184
2	180	AM	22.9 ± 2.84	3.36 ± 0.355	4.97 ± 0.994
		PM	10.6 ± 0.820	6.44 ± 2.35	7.49 ± 0.894
	450	AM	19.4 ± 3.65	2.23 ± 0.272	4.42 ± 2.46
		PM	10.6 ± 2.85	4.07 ± 1.079	8.31 ± 1.73

Table 5. Mean herbage catalpol concentration (mg/g DM) (±SEM) of plantain harvested in year 1 (2014/15) or year 2 (2015/16) after establishment at 450 kg N/ha/yr and 180 kg N/ha/yr fertiliser inputs harvested either in the morning (AM) or afternoon (PM).

Year	*N* fertiliser (kg/ha/y)	Time	Spring	Summer	Autumn
1	180	AM	0.286 ± 0.073	0.205 ± 0.020	0.003 ± 0.001
		PM	0.326 ± 0.019	0.118 ± 0.009	0.006 ± 0.001
	450	AM	0.257 ± 0.077	0.116 ± 0.032	0.005 ± 0.0
		PM	0.108 ± 0.014	0.390 ± 0.041	0.006 ± 0.001
2	180	AM	0.022 ± 0.008	0.012 ± 0.002	0.014 ± 0.004
		PM	0.015 ± 0.010	0.042 ± 0.013	0.011 ± 0.003
	450	AM	0.013 ± 0.005	0.013 ± 0.005	0.012 ± 0.002
		PM	0.043 ± 0.029	0.023 ± 0.003	0.011 ± 0.003

Discussion

Compound concentration

The concentration of secondary plant compounds observed in the current study was relatively low with average values of 2.02, 8.79 and 0.073 mg/g DM for aucubin, acteoside and catalpol, respectively. Previous studies documenting the concentration of secondary plant compounds in *P. lanceolata* have demonstrated large variation in the concentration. For example, aucubin concentrations up to 40 mg/g DM have been observed in naturalised American ecotypes (Bowers et al. 1992) while a concentration of 78 mg/g DM acteoside was recorded in naturalised Japanese ecotypes (Al-Mamun et al. 2008). However, the low values reported here are consistent with those of Gardiner et al. (2017) who compared wild ecotypes from Japan with commercial cultivars of New Zealand, cv Lancelot and cv. Ceres Tonic, revealing considerably lower secondary compound levels in the commercial cultivars.

Catalpol was only present in very low in concentrations (<1.0 mg/g DM). This is similar to previous findings (Tamura and Nishibe 2002; Navarrete et al. 2016). In a New Zealand study, Navarrete et al. (2016) found 'Ceres Tonic' plantain had almost nil concentration of catalpol. Furthermore, Tamura and Nishibe (2002) found no trace of catalpol in the cultivar 'Ceres Tonic' whereas the concentration of catalpol in the cultivar 'Grasslands Lancelot' fluctuated between 10 and 20 mg/g DM. Natural ecotypes of plantain can contain up to 12 mg/g DM catalpol (Gardiner et al. 2017). Catalpol is biologically synthesised from its precursor aucubin (Rayment and Higginson 1992). The low concentration of catalpol reported here and in other studies (Tamura and Nishibe 2002; Navarrete et al. 2016) suggests that the selection of the cultivar 'Ceres Tonic' has reduced the synthesis of catalpol from aucubin. This suggests that not all plantains are the same from a secondary plant compound perspective. Tonic appears to have uniquely lower secondary compound abundance which may be related to its suitability as a grazed forage (Stewart, 1996).

Seasonal changes

All of the secondary compounds showed seasonal differences in their concentrations, with aucubin and acteoside showing a similar seasonal distribution. The concentration of aucubin and acteoside appeared to peak in the first spring and was consistently lowest in summer. However, aucubin showed an annual change in concentration, with a lower concentration in spring of year 2 which may be related to plant age (Pankoke et al. 2013). For acteoside, there was no change in the seasonal concentration from year 1 to year 2. This may suggest that the seasonal influence on the presence of acteoside remains consistent after the first year of growth. Catalpol concentration peaked in the first spring and summer of growth and declined to very low concentration following summer of year 1 (>0.05 mg/g DM).

This seasonal distribution is different from that seen by Bowers et al. (1992) who reported an increase in aucubin and catalpol content over the growing season (spring to autumn). In their experiment, aucubin peaked in autumn with a concentration of around 40 mg/g DM. This is similar to the seasonal distribution reported by Tamura and Nishibe (2002) who found spring harvested plantain-contained concentrations of acteoside at 15 mg/g DM which increased to 41 mg/g DM in autumn.

The effect of treatments on secondary compound concentration may be affected by the morphological state of the plant and abundance of different tissue types. In plant defense chemistry, younger leaves or more vulnerable tissue such as meristems, often have higher concentrations of anti-herbivory properties (Pankoke et al. 2013). In the current study, acteoside was present in plantain in the greatest concentrations of the secondary compounds, and was the compound most sensitive to seasonal variation. Acteoside was present at concentrations from 60 to 90 mg/g DM in natural ecotypes of plantain (Fajer et al. 1992), and from 15 to 41 mg/g DM in the cultivar 'Ceres Tonic' (Tamura and Nishibe 2002) which is similar to the concentrations presented here. The largest concentration of acteoside was observed in spring when the plant is generally in a vegetative stage. The highest measured concentration of acteoside was recorded in AM samples in spring under 180 kg N/ha/yr (22–36 mg/g DM), which reflect a plant which is leafy and growing less actively than a well fertilised plant in the afternoon. Similarly, aucubin concentration was greatest in spring but less affected by time of day or N fertiliser rate.

N fertiliser rate

The results of this experiment suggest that N fertiliser rate has little influence on the presence of secondary compounds. There was a small, inconsistent N fertiliser influence on the concentration of catalpol in summer. However, regardless of the N fertiliser influence, the concentration of catalpol remained very low (>1 mg/g DM). When N fertiliser was applied at 450 kg N/ha/yr, the concentration of acteoside was lower than the 180 kg N/ha/yr treatment. However, this was only seen in spring of year 1. This negative relationship between N fertiliser and secondary compound concentration in plantain has been reported previously. Tamura (2001) showed the concentration of aucubin and acteoside were lower in plantain when N fertiliser was applied compared with those which received no N fertiliser.

Time of day effects

There was limited effect of harvesting herbage at different times of day on the concentration of aucubin and catalpol. The concentration of aucubin was unaffected by time of the day which herbage was harvested. An inconsistent time of day effect on catalpol concentration of plantain herbage. However, the change in catalpol concentration from morning to afternoon was relatively small (>0.2 mg/g DM). This suggests management strategies of harvest time, cannot be used to alter the concentration of aucubin and catalpol in plantain herbage.

In contrast, there was a strong effect of the time of day which plantain herbage was harvested on the concentration of acteoside in spring of both years. Morning harvested plantain had almost twice the concentration of acteoside than afternoon harvested plantain. The mechanisms behind a diurnal decrease in acteoside concentration are unclear and may be indicative of errors in the quantification of metabolites rather than a diurnal effect *per se*. Previous work by Tamura (2001) found that the concentration of acteoside was lower for plantain grown in the shade than for plantain in sunlight, and they concluded that shade represses the growth and accumulation of aucubin and acteoside in plantain. Therefore, it was assumed plantain harvested in the morning may have had a lower acteoside concentration than that harvested in the afternoon. However, this was not the case in this experiment and may be related to differences in environmental factors as their experiment was carried out in Japan.

Conclusions

This study showed the three secondary compounds were present in plantain herbage with the greatest concentration for acteoside (range 36.0–1.77 mg/g DM), intermediate for aucubin (range 5.85–0.566 mg/g DM) and lowest for catalpol (>0.390 mg/g DM). While seasonal fluctuations were evident, the effect of management strategies, harvesting in the morning or afternoon and altering N fertiliser rate had little effect on the concentration of all compounds. Based on this observation, there appears to be little opportunity to use N fertiliser or time of harvest to alter the secondary compounds in plantain herbage as a potential environmental mitigation option.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was undertaken as part of the Forages for Reduced Nitrate Leaching program, with principal funding form the New Zealand Ministry of Business, Innovation and Employment. The program is a partnership between DairyNZ, AgResearch and Landcare Research, Plant and Food, Lincoln University and Foundation for Arable Research.

References

Al-Mamun M, Abe D, Kofujita H, Tamura Y, Sano H. 2008. Comparison of the bioactive components of the ecotypes and cultivars of plantain (*Plantago lanceolata* L.) herbs. Animal Science Journal. 79(1):83–88.

Andary C, Wylde R, Laffite C, Privat G, Winternitz F. 1982. Structures of verbascoside and orobanchoside, caffeic acid sugar esters from *Orobanche rapum-genistae*. Phytochemistry. 21(5):1123–1127.

Bourgaud F, Gravot A, Milesi S, Gontier E. 2001. Production of plant secondary metabolites: a historical perspective. Plant Science. 161(5):839–851.

Bowers MD, Collinge SK, Gamble SE, Schmitt J. 1992. Effects of genotype, habitat and seasonal variation on iridoid glycoside content of *Plantago lanceolata* and the implications for insect herbivores. Oecologia. 91:201–207.

Box LA, Edwards GR, Bryant RH. 2016. Milk production and urinary nitrogen excretion of dairy cows grazing perennial ryegrass-white clover and pure plantain pastures. Proceedings of the New Zealand Society of Animal Production. 76:18–21.

Box LA, Edwards GR, Bryant RH. 2017. Milk production and urinary nitrogen excretion of dairy cows grazing plantain in early and late lactation. New Zealand Journal of Agricultural Research. 60(4):470–482.

Chen CH, Lin YS, Chien MY, Hou WC, Hu ML. 2012. Antioxidant and antihypertensive activities of acteoside and its analogs. Botanical Studies. 53(4):421–429.

Cheng L, Judson HG, Bryant RH, Mowat H, Guinot L, Hague H, Taylor S, Edwards GR. 2017. The effects of feeding cut plantain and perennial ryegrass-white clover pasture on dairy heifer feed and water intake, apparent nutrient digestibility and nitrogen excretion in urine. Animal Feed Science and Technology. 229(Supplement C):43–46.

Darrow K, Bowers MD. 1999. Effects of herbivore damage and nutrient level on induction of iridoid glycosides in *Plantago lanceolata*. Journal of Chemical Ecology. 25(6):1427–1440.

Deaker JM, Young MJ, Fraser TJ, Rowarth JS. 1994. Carcuss, liver and kidney characteristics of lambs grazing plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*), white clover or perennial ryegrass. Proceedings of New Zealand Society of Animal Production. 120:51–61.

Fajer ED, Bowers MD, Bazzaz FA. 1992. The effect of nutrients and enriched CO_2 environments on production of carbon-based allelochemicals in plantago: a test of the carbon/nutrient balance hypothesis. The American Naturalist. 140(4):707–723.

Gardiner CA, Clough TJ, Cameron KC, Di HJ, Edwards GR, de Klein CAM. 2016. Potential for forage diet manipulation in New Zealand pasture ecosystems to mitigate ruminant urine derived N_2O emissions: a review. New Zealand Journal of Agricultural Research. 59(3):301–317.

Gardiner CA, Clough TJ, Cameron KC, Di HJ, Edwards GR, de Klein CAM. 2017. Potential inhibition of urine patch nitrous oxide emissions by *Plantago lanceolata* and its metabolite aucubin. New Zealand Journal of Agricultural Research. 1–9.

Kato Y. 1946. Mechanism of uric acid excretion stimulation by aucubin. Folia Phamacol. 42:37–40.

Martin KM, Edwards GR, Bryant RH, Hodge M, Moir J, Chapman DF, Cameron KC. 2017. Herbage dry-matter yield and nitrogen concentration of grass, legume and herb species grown at different nitrogen-fertiliser rates under irrigation. Animal Production Science.

Navarrete S, Kemp PD, Pain SJ, Back PJ. 2016. Bioactive compounds, aucubin and acteoside, in plantain (*Plantago lanceolata* L.) and their effect on in vitro rumen fermentation. Animal Feed Science and Technology. 222:158–167.

O'Connell CA, Judson HG, Barrell GK. 2016. Sustained diuretic effect of plantain when ingested by sheep. Proceedings of the New Zealand Society of Animal Production. 76:14–17.

Olsen SR, Cole CV, Watanabe FS. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Washington: USDA.

Pae HO, Oh GS, Choi BM, Shin S, Chai KY, Oh H, Kim JM, Kim HJ, Jang SI, Chung HT. 2003. Inhibitory effects of the stem bark of *Catalpa ovata* G. Don. (bignoniaceae) on the productions of tumor necrosis factor-alpha and nitric oxide by the lipopolisaccharide-stimulated RAW 264.7 macrophages. Journal of Ethnopharmacology. 88(2–3):287–291.

Pankoke H, Buschmann T, Muller C. 2013. Role of plant beta-glucosidases in the dual defense system of iridoid glycosides and their hydrolyzing enzymes in *Plantago lanceolata* and plantago major. Phytochemistry. 94:99–107.

Rayment GE, Higginson FR. 1992. Australian laboratory handbook of soil and water chemical methods. Melbourne: Inkata Press Pty Ltd. xvii + 330pp. p.

Rumball W, Keogh RG, Lane GE, Miller JE, Claydon RB. 1997. "Grasslands lancelot" plantain (*Plantago lancelata* L. New Zealand Journal of Agricultural Research. 40(3):373–377.

Stewart AV. 1996. Plantain (*Plantago lanceolata*) – a potential pasture species. Proceedings of the New Zealand Grassland Association. 58:77–86.

Tamura Y. 2001. Effects of temperature, shade, and nitrogen application on the growth and accumulation of bioactive compounds in cultivars of *Plantago lanceolata* L. Japanese Journal of Crop Science. 70(4):548–553.

Tamura Y, Nishibe S. 2002. Changes in the concentration of bioactive compounds in plantain leaves. Journal of Agricultural and Food Chemistry. 50:2514–2518.

Watkinson JH, Kear MJ. 1994. High performance ion chromatography measurement of sulfate in 20 mM phosphate extracts of soil. Communications in Soil Science and Plant Analysis. 25(7-8):1015–1033.

Zhou YC, Zheng RL. 1991. Phenolic compounds and an analog as superoxide anion scavengers and antioxidants. Biochemical Pharmacology. 42(6):1177–1179.