The Effect of Diverse Pasture and Italian Ryegrass on Urine Patch Size and Plant Nitrogen Uptake

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The intensification of the dairy industry in New Zealand has prompted the use of fertiliser and legumes to produce productive pastures with high nitrogen content. High quality pastures create an imbalance between nitrogen supply and animal demand, the N taken up by pasture species and utilised for animal products eg. meat and milk, the excess N (75-95%) returns to the soil in excrement, especial urine. Urine N is a major source of N loss in pastoral farming systems. The N load onto pastures is a function of urine volume, urinary N concentration, urination frequency, cow density and the urine patch area, the plant uptake following this indicates the overall loss. Diverse pastures and pastures containing Italian ryegrass are increasingly being used in dairy pastures to improve dry matter production and potentially reduce N loss, however there is little information on the effect of these pastures on the urine patch area (a function on N loading) and plant uptake over winter.

The trial investigated the effect of diverse pasture mixes and those containing Italian ryegrass on the area of urine patches using thermal imaging technology to record the 'wetted' area of urination events. There was no significant effect of species diversity and the presence of Italian ryegrass on urine patch size. There urine patch size was significantly higher in the morning compared to the afternoon (0.35 v's 0.16m^2 ±0.03 respectively). This effect occurred because of higher urine volume and pasture cover. Calibration curves were generated to determine urine volume from a certain patch area. A clear increase in urine area in short pasture was shown, however the range of urine volume generated was higher than results obtained from urine sensor harnesses. The plant N uptake was significantly higher inside urine patches than outside (143 v's 62 kgN/ha respectively), a result of a two-fold increase in pasture yield and increased N content in pasture. The overall N loading per ha inside urine patches ranged between 16.5 and 23.1kgN/ha, and an excess N after N uptake between 12.2 and 17.9kgN/ha.

The increase in urine patch size in the morning compared to the afternoon is thought to be a function of increased urine volume that occurs through diurnal variation and the pasture height which had been grazed for much longer during the morning measurements. The higher N uptake inside the urine patches occurs because of the higher N input and supply to plants, resulting in higher growth rates and DM accumulation, and also higher N% in the plant tissue. Combined there was not a large difference in the N loss between treatments, a major factor influencing this was the stock density on the pastures based on DM cover. There needs to be further research into the diurnal variation ion urine patches, along with variation in urine volume and N concentration the N loading onto a soil can be better understood.

Keywords: Diverse pasture*, Lolium multiflorum*, Nitrogen, N Loading, Urine Patch, DM accumulation N Uptake.

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Chapter 1

Introduction

The New Zealand dairy industry plays an important role in New Zealand's economy as it is the largest contributor of agricultural export. Dairy products contribute 37% of primary industry exports and 29% of total New Zealand merchandise exports with an export revenue of $13.2 billion in 2014-15 (NZIER November 2015). The farming systems in New Zealand are predominantly pasture based with animals grazing outdoors year round, the milk production and farm profit is closely related to the DM produced and consumed by the cows (Chapman *et al* 2008). By increasing the DM harvested by 1t DM/ha can increase the gross margin by $339/ha (Savage and Lewis 2005). This has prompted significant intensification of farming systems through the use of irrigation and nitrogen fertiliser to produce highly productive pastures which allow higher stocking rates to increase the DM consumption/ha (Mackinnon *et al* 2010). Intensification also brings with it issues for the dairy industry as the utilisation of the N and P in the system is poor, creating an impact on the environment such as nutrient enrichment of ground/surface water and contribution of greenhouse gases (CH_4^+, N_20) (Monaghan *et al* 2010, Di and Cameron 2002a).

Perennial Ryegrass (*Lolium perenne* L.) and White Clover (*Trifolium repens*) is the most common pasture species grown in dairy pastures in New Zealand. These species are used due to the suitablility of the temperate climate and the ability to produce high quality feed year round. The potential production of this pasture is limited during dry summer periods which impact on the dry matter production and persistence of the species. The use of alternative grass, clovers and legumes in diverse pasture mixtures is becoming increasingly more common. Herbs such as Chicory (*Cichorium intybus*) and Plantain (*Plantago lanceolata*) are included to increase the total annual dry matter production, by increasing spring DM production and extending the summer pasture production (Nobilly et al. 2013). Lucerne (*Meticago sativa*) is an alternative legume used in pasture mixes to improve the feed value, fix nitrogen in the soil and provide high dry matter production in dry periods due to its long taproot system (Brown *et al* 2000, 2003, Moot *et al* 2003).

The intensification of dairy system in New Zealand has prompted the use of fertiliser and legumes to produce productive pastures with high N content. In grazed farming systems N taken up by pasture species and utilised for animal products eg. meat and milk, but the majority of N ingested (75-95%) returns to the soil in excrement, especially in urine (Eckard *et al* 2010, Selbie 2014). The urinary N is not spread evenly but concentrated in patches of which the N exceeds the plant requirements, therefore urine patches have been identified as a major source of N loss (Di and Cameron 2002a). The total paddock soil N loading from urine patches is not just about the concentration of N in the urine but also the coverage area of urine patches. The area of a single urine patch is affected by factors such as the volume of the urination event (L) and the height of the pasture. At farm level, there is a factor of urine patch distribution, urine patch overlap, the timing of urination (related to climatic condition during and after) and the number of times a paddock is grazed during different times of the year (Haynes and Williams 1993, de Klein 2001, Di and Cameron 2002, Romera *et al* 2012).

There are both animal and plant factors that can affect the amount of N that is not only applied to the soil but also taken up. The cow factors that determine the soil N loading are the concentration of nitrogen in the urine, the variation in frequency and volume of urination events, and the time of day the urination events occur (Romera *et al* 2012). There is significant knowledge and information on the concentration of N in cow urine, however the factor of volume, frequency and coverage of urine events have only recently focused on. The urine patch coverage was done on urine patches in winter forage crops (Ravera 2014), there is little information on patch coverage in pastures. The quality and nutritive characteristics of pastures will influence the amount of nitrogen in the diet, and therefore the utilisation and concentration of N in the urine. Plants will utilise the N deposited in urine, therefore the rate of pasture growth inside the urine patch, the amount of DM accumulated and the crude protein (CP) content of the pasture following the urination event will determine the N uptake. Due to high N leaching risk during the winter, inclusion of Italian ryegrass, which has high herbage production during winter/early spring, may present an opportunity to improve plant N uptake over winter.

The aim of this dissertation was to identify how pastures' containing Herbs and Italian Ryegrass influence urine patch area and what affect the urine event has on the yield, regrowth and persistence of plant species in the urine patches.

Chapter 2
Literature Review

2.1 Nutritive Value of Pasture Species

2.1.1 Dry Matter Production of Pasture Species

The trial is investigating the effect of a diverse pastures and pastures containing Italian ryegrass on the urine patch size and the pasture growth and uptake of nitrogen under a urine patch. The diverse pastures contain additional herb and legume species; chicory, plantain and Lucerne, compared to simple perennial ryegrass white clover pastures. The pasture quality is an important factor in pastures, the quality characteristics indicate how much nitrogen is contained in the pasture which affects nutrient intake and therefore the excretion of nitrogen in urine patches. This is important when looking at the overall N loading onto a soil.

Perennial Ryegrass

Perennial Ryegrass (*Lolium perenne* L.) is the most widely used temperate grass species in New Zealand (Charlton and Stewart 1999), and is a major dietary component in the intensive livestock grazing farm systems (Easton *et al* 2001). It grows in a wide range of fertile soils, is easy to establish and manage, has a rapid recovery from hard grazing and produces high quality forage. It requires adequate soil moisture and performs poorly in hot, dry seasons where many other deep rooted species maintain production (Charlton and Stewart 1999, Easton *et al* 2001). The annual dry matter (DM) production of perennial ryegrass in New Zealand ranges between 9 – 20 tonnes DM/ha/yr (Easton *et al* 2001, DairyNZ 2012). The large variation in production exists between regions and is determined mainly by the temperature, grazing management, and soil moisture and fertility (Stewart *et al* 2014). There are significant changes in the seasonal production and morphology of perennial ryegrass pastures based largely on temperature and therefore the region it is grown. The use of irrigation increases dry matter production, by 13% in Waikato (Clark *et al* 2010) and 80% in Canterbury (Mcbride, 1994). Dry matter production of perennial ryegrass is lowest during winter.

White Clover

White Clover (*Trifolium repens*) is a legume commonly sown in a mix with Perennial ryegrass in New Zealand. It's key benefits inlcude; N fixation as a legume, improved sward quality, complements seasonal growth pattern of common grass species, and improves forage intake and utilisation rates of animals (Caradus *et al* 1996). The ability to compliment perennial ryegrass growth and fix nitrogen in the soil provides a major benefit to New Zealand farming systems which typically graze animals outdoors year round. White clover is a summer active clover with an optimal growing temperature

5°C higher than that of perennial ryegrass (Hoglund and Brock 1987, Brock *et al* 1989), however production and persistence can be limited in very dry summer conditions. White clover grows well in moderate to highly fertile soils (Stewart *et al* 2014), but can be successfully established in a range of soil types throughout New Zealand. White clover is usually sown in a mixed pasture sward with grass, legume and herb species. Grass species create competition against clover for plant resources, especially light, thus white clover is likely to stabilise at around 20% of the total dry matter production in a mixed pasture (Andrews *et al* 2007). The dry matter production of white clover varies significantly in a pasture depending on the pasture management and climate (rainfall), for example Brougham (1960) reported less than 1tDM/ha for white clover and Ball *et al* (1978) found clover DM yields between less than 1tDM/ha up to 7tDM/ha. In a pure sward white clover can produce 10-12tDM/ha (Brock 1973). The nitrogen fixed by clover in a mixed sward is likely to be similar to that of pasture receiving 200 kg DM/ha (Andrews *et al* 2007).

Italian Ryegrass

Italian ryegrass (*Lolium multiflorum*) is an erect, annual rye grass. Italian ryegrass has improved cool season growth over other ryegrass species, therefore it is commonly sown to fill winter/early spring feed shortages or ensiled for use as a supplement feed (de Ruiter 2007). Italian ryegrass is typically sown in autumn and due to winter growth can be grazed 2-3 times before spring. Italian ryegrass is also sown in mixed pastures to improve cool season growth and spread the annual pasture production more evenly. Italian ryegrass has rapid establishment (Ryan-Salter 2011, Stewart *et al* 2014), in good growing conditions it is ready for a light grazing 4-6 weeks after sowing (perennial ryegrass = 6-8 weeks). Flowering and seed set occurs in early summer after which production is limited, particularly in summer dry areas. In summer moist areas or under irrigation some Italian ryegrass cultivars may persist and maintain high production in a second winter (Ryan-Salter 2011) as long as insect pressure is low (Stewart *et al* 2014). The annual DM production in Italian ryegrass monocultures has shown variable results. Ryan- Salter and Black (2012) reported a total DM production of 9.75tDM/ha/yr and was supplemented with irrigation once in summer. All NZ trials by the NZ Plant breeders association (NZPBRA, 2015) reported an annual DM production ranging between 13 and 18.5tDM/ha/yr, and when sown in March as a winter feed crop produced between 7-8tDM/ha in 6-8 months. Moot *et al* (2007) reported a yield of 14.3tDM/ha/yr in Marbella Italian ryegrass. The DM yield is higher in a pasture mix with legumes. Ryan-Salter and Black (2012) found a mix of Italian ryegrass and red clover, and Italian ryegrass with both red and balansa clover produced 41% and 35% respectively more DM than a monoculture of Italian Ryegrass.

Plantain

Plantain (*Plantago lanceolata*) is a perennial herb that is sown as a monoculture, in diverse pasture mixes and/or mixtures with clover (Lee *et al* 2015). Plantain establishes quickly, is responsive to nitrogen and grows well in a wide range of soil types. However it is very sensitive to competition in the early stages of establishment (Stewart 1996). Plantain has a relatively high growth throughout summer and is also winter active depending on the cultivar. The species is tolerant to drought conditions and many common pests and diseases (Stewart 1996, Ivins 1952) and is a preferable species for dryland pasture mixes to improve summer dry matter production. Plantain tends to be highly palatable and selectively grazed though, palatability declines as it matures and starts producing reproductive stems (Ivins 1952, Derrick *et al* 1993). The annual DM yield for a pure plantain sward near Canterbury, was approximately 7.6-8.4tDM/ha/yr (Stewart 1996) in, showing significantly increased yield during summer months. Lee *et al* (2015) reported higher DM yield between 9.8 and 13.7tDM/ha/yr in Waikato, also showing high summer production which varied with grazing interval and grazing residual. Pasture-based plantain mixes can yield as high as 20tDM/ha/yr (Moorhead and Piggot 2009). In a mixed sward with white clover Rumball *et al* (1997) reported a yield of 11.7tDM/ha/yr, plantain consisted of 64% of the DM produced (approximately 7.5tDM/ha). Plantain yield varied significantly with the type of grazing, the yield was 27% higher with less frequent grazing. Plantain is sown in diverse mixtures with grasses to improve summer DM production., Moorhead and Piggot (2009) reported significant yield differences in plantain-based pastures and perennial ryegrass-based pastures in summer (1.8 t DM/ha) and autumn (0.9 t DM/ha). Competition in mixed swards is increased by the competitive ability of grass species, especially perennial ryegrass. The plantain content in a mixed sward tends to decrease with time, sown for 3 years in a mix with perennial ryegrass the plantain content was around 10%, and in a clover mix it was higher at 30% (Rumball *et al* 1997). There is evidence that shows plantain is tolerant to repeat and severe grazing (Ivins 1952), however this can also lead to a high proportion of bare ground that can reduce its competitiveness in mixed swards.

Chicory

Chicory (*Cichorium intybus*) is a perennial leafy herb, typically sown in diverse pasture mixes for finishing young stock especially lambs and deer. It has a deep taproot that grows well in fertile, free draining soils. The ability to attain more water, nutrients and minerals (taproot) means chicory can produce high yields of high quality forage in spring and summer (Charlton and Stewart 1999, Li and Kemp 2005, Caradus *et al* 2013). Chicory is dormant (little growth) in winter, requiring a vernalisation period to produce seed in order to persist (Lee *et al* 2015). It can be sown in spring or autumn and establishes relatively quickly, growth is very rapid in spring and early summer causing early maturation and reproductive stem formation (Li and Kemp 2005). It must be grazed to maintain a vegetative state to maximise persistence, feed production and feed quality (stem:leaf). Chicory can be grazed closely but not frequently (set stocking) so benefits from rotational grazing (Clark *et al* 1990, Li *et al* 1997c).

DM production levels of pure chicory swards range between 8.5-9.4tDM/ha/yr (Li *et al* 1997a), in some research the yield was significantly higher at 19tDM/ha/yr in pure dryland swards (Brown and Moot 2004). A mixed sward with Perennial ryegrass and White clover the yield ranged between 16.2 and 12.6tDM/ha/yr, chicory contributed between 22 and 87% of the dry matter (Hume *et al* 1995). The decline in plant density of chicory over time is largely affected by grazing, especially during late spring and autumn. There is a flush of growth during autumn before it is relatively dormant throughout winter; during this period chicory is sensitive to hard selective grazing and stock treading (Li and Kemp 2005). Chicory to be very sensitive to 'pulling' during grazing, if it is grazed before the root system has fully developed (enough to hold plant in soil) losses can be up to 12.9% at first grazing 19 weeks after sowing (Powell *et al* 2007).

Lucerne

Lucerne (*Meticago Sativa L.*) is a perennial legume species that produces high quality forage for livestock. Lucerne is a drought tolerant plant with a long tap root, it can draw water from deeper in the soil profile than other shallow rooted species such as perennial ryegrass and white clover. Trials have shown lucerne roots attaining water as deep as 2.3m, which is deeper than other tap rooted species such as chicory and red clover (1.9m) (Brown *et al* 2005). The summer growth of lucerne is not as compromised by water stress, along with the ability to fix N (average of 160kgN/ha/yr) lucerne can provide a large quantity of palatable feed for livestock in dryland environments (Bennett, 2012). Lucerne growth increases in spring, peaks in summer and slows in autumn (Mcgowan *et al* 2003, Brown et al 2005). The winter growth of lucerne is low as it becomes dormant, growth slows after frosts occur. The growing point of leaves and nodes occurs at the tips of stems; and new stems grow from the crown at the base of the plant. Grazing management is key as the plant regrowth is hindered if the crown is damaged, as new buds must form. Lucerne must be rotationally grazed and cannot be set stocked or grazed hard (Moot *et al* 2003).

Lucerne had a mean yield of 20tDM/ha over 5 years in a dryland environment in Lincoln (Brown et al 2003), under irrigation the yield was significantly higher in the first year at 29tDM/ha but decreased to 17tDM/ha/yr in the fifth year (Brown *et al* 2000). A trial at Ashley Dene farm in Lincoln showed Lucerne under dryland conditions had much lower yields, ranging between 11.4 and 12.9tDM/ha/yr (Bennett 2012), however this was only trialled for one year. Ryegrass has similar yields to lucerne under irrigation, however the feed quality is lower in late spring/summer as ryegrass is in its reproductive stage. The DM production of lucerne based diverse pasture under dryland conditions ranged between 6.8 – 12.4tDM/ha/yr in Dashwood and 3.8 – 10tDM/ha/yr in Winchmore over three years (Daly *et al* 1996). Growth was higher than a perennial ryegrass/white clover mix in spring and summer months especially, lucerne pasture performed consistently well and maintained a high legume content (as it is a legume). The persistence of lucerne depends on its ability to store root reserves (soluble carbohydrates), this occurs throughout the growing season especially during autumn flowering, these become available in spring for growth after dormancy (Brown *et al* 2004). The grazing management is very important for the persistence of lucerne, and if done correctly lucerne can remain a highly productive crop for more than five years. Brown *et al* (2003) found lucerne to be significantly more persistent than other tap root forages (chicory and red clover), after 5 years lucerne still consisted of 94% of dry matter whilst chicory and red Clover consisted of 61% and 0% respectively.

2.1.2 Diverse v's Simple DM production

In an attempt to capture the yield benefits, these species are sown in together in diverse mixtures containing multiple pasture species. The annual dry matter production of diverse pastures is higher than standard perennial ryegrass/white clover pasture, both under irrigation and on dryland pasture. Nobilly *et al* (2013) investigated the productivity of standard ryegrass, high sugar ryegrass and tall fescue both as binary swards with white clover and in diverse pastures with herbs, lucerne and red clover under irrigation. The results showed diverse pastures had a higher overall dry matter (DM) production of 16.77tDM/ha/year compared to simple pastures at 15.15tDM/ha/year. The results show diverse pasture mixes increased DM production in all seasons except autumn, but was only significant in summer. A similar trial comparing standard ryegrass to comprehensive, Lucerne and red clover based mixtures under dryland conditions showed comparable results with significant DM increases in summer (Daly *et al* 1996). The increase in DM production from diverse pastures predominantly occurred from improved production throughout spring and summer, this is highlighted especially in the pastures under dryland conditions (Daly *et al* 1996) with significant increases in production over these months. The results under irrigation (Nobilly *et al* 2013) show less significant changes in the DM production from diverse pastures (as water is not limiting), however there is a significant increase in DM production shown in the summer of 2011. Increases summer production in diverse pastures can be explained by the abundance of herbs (chicory and plantain) which grow rapidly at this time of year given adequate water (Sanderson *et al* 2005). The presence of chicory (possibly plantain), lucerne and red clover contribute to increased DM production due to their deep tap roots that increase the amount of soil water available to the plant (Brown *et al* 2006). The increase in DM production in winter was not significant under irrigation (Nobilly *et al* 2013), however there was no addition of italian Ryegrass in the mix which has been shown to have higher winter growth than other herb and legume species.

Persistence of species in a diverse pasture mix is variable, some are more susceptible to competition than others over time. A pasture mix has some species more vigorous than others, for example perennial ryegrass is a very competitive grass species during establishment, clovers and herbs are less competitive. There needs to be careful grazing management of a mixed sward and knowledge of if, and when a species requires setting seed to persist in the sward over time, if species cannot the abundance of species in the mix drops quickly over time. Daly *et al* (1996) investigated the change in botanical composition of mixed pasture swards and standard ryegrass/white clover pastures over three years at two different sites. In all treatments, the grass content increased, the legume percentage decreased over time in all except the lucerne based treatment (as it is a legume) and the percentage of herbs remains similar around 15-30% (with an increase in the red Clover based mix). This showed that over time grass becomes increasingly more dominant, however further time gathering data is required to determine the long-term persistence of the pasture species.

Table 2.1.1: Percentage contribution of herbage components to production at Dashwood and Winchmore over a 3 year period (1992-95). (Daly et al 1996).

		Dashwood			Winchmore		
Component	Treatment	1992–93	1993–94	1994–95	1992–93	1993–94	1994–95
Grass	Ryegrass standard	51.5	45.4	67.4	60.2	63.9	72.5
	Comprehensive MSP	17.2	19.8	41.6	24.6	38.8	58.1
	Lucerne MSP	16.8	12.7	27.6	22.5	34.9	37.0
	Red clover MSP	28.4	17.6	34.1	29.9	38.9	50.2
	LSD[5]	7.1	7.2	18.0	4.8	6.3	10.8
Legume	Ryegrass standard	28.4	41.1	16.8	34.4	28.5	15.7
	Comprehensive MSP	52.7	52.6	33.0	28.9	37.6	16.1
	Lucerne MSP	35.1	50.1	40.5	34.6	30.7	21.0
	Red clover MSP	41.7	44.6	18.0	31.8	39.8	17.8
	LSD[5]	9.2	19.5	16.6	8.1	7.8	7.2
Herb	Ryegrass standard	0.0	0.0	0.0	0.0	0.0	0.0
	Comprehensive MSP	17.2	20.8	13.1	15.3	18.3	19.8
	Lucerne MSP	29.8	31.1	17.8	24.0	28.8	36.6
	Red clover MSP	14.3	30.8	42.2	16.6	15.4	24.6
	LSD[5] (only for MSP comparison)	8.3	21.3	23.5	6.1	8.4	5.3

2.2 Nutritive Value of Pasture Species

2.2.1 Perennial Ryegrass

The nutritive value of perennial ryegrass to grazing animals is variable depending on the season, maturity and management practices (particularly grazing) (Burke *et al* 2002b). The ME content ranges between 9.9-11.8MJME/kgDM (Table 2.2.1), the ME is shown to be the highest in winter and lowest in summer (11 and 9.9MJME/kgDM respectively) (Fulkerson *et al* 2007). The crude protein levels in previous research ranges between 12-26% (Table 2.2.1). CP concentrations in forage must exceed 10% of DM for livestock maintenance and about 19% of DM for high-producing dairy cows or young growing stock (Waghorn and Clarke 2004).

The protein levels in ryegrass vary significantly, however pastures typically contain a legume or herb species to increase protein levels to acceptable levels. The CP levels are highest in the spring and are significantly lower in the summer and autumn (20-30% DM v's <20% DM), also the crude protein levels reduce with increasing plant maturity (Burke *et al* 2002b). This explains some variation in results possibly taken in different seasons or are the mean for the whole year. The trial by Fulkerson (2007) found the CP levels were the highest in spring pasture and the lowest in autumn (Table 2.2.1). This trial was done in the temperate climate of Australia which is a similar climate to New Zealand.

Table 2.2.1 Nutritive Value of Perennial Ryegrass (*Lolium perenne*)

Author	Herbage Quality (% Dry Matter)				
	CP[1]	NDF[2]	ADF[3]	WSC[4]	ME (MJME/kgDM)[5]
<u>New Zealand</u>					
Rattray *et al* 1974	3.28*	32.07	28.08	12.62	-
Fraser and Rowarth 1996	20.1	44.7	20.2	-	-
Jackson *et al* 1996	4.5*	40.6	19.4	7.4	-
Harris *et al 1998*					
December	9.3	61.3	33.2	9.7	10.4
April	20.5	52.7	28.4	8.0	10.2
Barrell *et al* 2000	22.5	48.3	22.9	8.1	11.8
Burke *et al* 2000 & 2002b	15.5	48.7	25.5	9.1	11
Burke *et al* 2002a	15.5	48.0	-	12.1	10.1
Harrington *et al* 2006	23.2	-	28.1	-	-
Hutton *et al* 2010	12.3	36.2	20.3	-	10.55
<u>Australia</u>					
Fulkerson *et al* 2007					
Autumn	24.0	49.7	26.6	5.3	10.0
Spring	26.3	55.2	25.9	6.3	10.6
Summer	24.3	51.5	31.3	5.7	9.9
Winter	24.3	48.9	23.2	7.8	11.0

[1] Crude Protein [2] Neutral Detergent Fibre [3] Acid Detergent Fibre

[4] Water Soluble Carbohydrates [5] Metabolisable Energy

* Total Nitrogen % in Dry Matter

2.2.2 Italian Ryegrass

The ME value of diploid italian ryegrass cultivars has limited research. The ME value of italian ryegrass is relatively high during winter, ranging between 12.2 and 13.3 MJME/kgDM (de Ruiter *et al* 2007). This is higher than perennial ryegrass, however it does not consider the change in ME of the pasture during spring, summer and autumn. Ryan-Salter and Black (2012) reported an average ME value of 11.4 for Italian ryegrass. This figure has a greater accuracy for ME, taking into account the decrease in ME (late summer) that dropped to 9.5% and 10.9% in February and March respectfully. The crude protein content is similar between much of the research, predominantly ranging between 19% and 23% (Table 2.2.2).

Ryan-Salter and Black (2012) reported a lower level of 15.4%, the CP levels of italian Ryegrass varied significantly at different times of the year. They are at the highest level in May and July (28.7% and 24.3% respectively) and reduce considerably during October, December and February (9.0%, 5.5% and 9.6% respectively) (Ryan-Salter and Black 2012). The WSC content ranges between 11% and 19% in cool climates, Hopkins (2003) showed a WSC content of 6.5% in a warm climate. The seasonal variation of WSC in ryegrass is influenced by temperature (Smith 1973, Kobayashi 2008), cooler temperatures increase WSC content due to the 'cold acclimation' mechanism in plants (the accumulation of metabolites such as the carbohydrate fructan). This would indicate higher levels of WSC during winter months, shown by results from Hopkins (2003) which showed a higher WSC content in Italian ryegrass in the cold climate (Table 2.2.2).

Table 2.2.2 Nutritive Value of Italian Ryegrass (*Lolium multiflorum*)

Author	Herbage Quality (% Dry Matter)				
	CP[1]	NDF[2]	ADF[3]	WSC[4]	ME (MJME/kgDM)[5]
New Zealand					
de Ruiter *et al* 2007					
Early Winter	18.2	22.6	14.2	11.5	13.3
Mid Winter	19.6	38.3	20.6	18.3	12.6
Late Winter	19.9	46.2	23.3	19.4	12.2
Ryan-Salter and Black 2012	15.4	-	-	-	11.4
USA					
Lippke and Ellis 1997	22.8	37.5	21.7	-	-
Canada					
Thompson *et al* 1992					
Autumn	19.6	50.7	35.6	-	-
Winter	20.2	44.6	33.5	-	-
South Africa					
Hopkins 2003					
Warm Regime	21.4	50.2	29.2	6.5	-
Cold Regime	19.7	51	26.3	15	-

[1] Crude Protein [2] Neutral Detergent Fibre [3] Acid Detergent Fibre

[4] Water Soluble Carbohydrates [5] Metabolisable Energy

2.2.3 White Clover

White clover has a higher feed value than perennial Ryegrass, the ME value ranges between 11-12MJME/kgDM in the previous New Zealand research (Table 1.2.3), hence its importance in New Zealand pastoral systems. As a legume, the crude protein content (and total N) in white clover is higher than that of perennial ryegrass ranging between 15-30% compared to 12-26% (Table 2.2.3 and 2.2.1). The crude protein content is highest in winter and spring when clover is in a vegetative (stoloniferous) growing state, the feed quality does not significantly reduce during seed production and remains high year round (Stewart *et al* 2014). Elevated crude protein levels indicate a high nitrogen level in the clover and therefore in the pasture, if N concentration is too high and exceeds ruminant livestock requirement high urine N deposits and N losses can occur in the soil. The WSC content in white clover range between 4.1% and 14.5%, which is similar to perennial ryegrass. The effect of temperature on the level of WSC is the same 'cold acclimation' mechanism as ryegrass, hence the WSC was higher in winter (Fulkerson *et al* 2007).

Table 2.2.3 Nutritive value of White Clover (*Trifolium repens*)

Author	Herbage Quality (% Dry Matter)				
	CP[1]	NDF[2]	ADF[3]	WSC[4]	ME (MJME/kgDM)[5]
New Zealand					
Rattray *et al* 1974	4.14*	38.86	25.75	7.94	-
Fraser and Rowarth 1996	28.0	23.2	18.0	-	-
Barrell *et al* 2000	29.8	26.6	20.1	10.2	12.2
Harris *et al* 1998					
December	21.5	37.6	27.8	8.1	11.7
April	24.6	36.1	23.7	9.4	10.7
Burke *et al* 2000 & 2002b	15.0	25.6	19.0	12.1	11.5
Burke et al 2002a	27.9	26.4	-	14.5	11.8
Harrington *et al* 2006	27.0	-	12.9	-	-
Australia					
Fulkerson *et al* 2007					
Autumn	-	-	-	-	-
Spring	28.1	33.9	23.3	7.5	9.3
Summer	24.2	27.6	21.0	4.1	10
Winter	29.8	29.1	19.3	9.0	10.5

[1] Crude Protein [2] Neutral Detergent Fibre [3] Acid Detergent Fibre
[4] Water Soluble Carbohydrates [5] Metabolisable Energy

2.2.4 Plantain

The nutritive value of plantain is relatively low, the ME is between 9.3% -10.7% (Table 1.2.4), the values are from Australian research and may differ from New Zealand results due to a differing growing environment. The ME value for plantain in New Zealand may differ. The CP content of plantain ranges from 14-28% in previous research (Table 2.2.4), on average it is higher than perennial ryegrass, but lower than white clover CP content. The CP content reduces with leaf maturation, which is related to the grazing interval. Lee *et al* (2015) found the CP content of Plantain declined with increasing grazing interval, this was correlated to extended leaf height which was higher and more mature when left ungrazed for longer intervals. The WSC content was very low at 3.5% in the autumn and 3.6% in spring (Fulkerson *et al* 2007), this increased to 14.6% in winter which has been a similar trend in herb, grass and legume species. The New Zealand research showed higher WSC levels between 14 and 17% (Table 1.2.4), but it is a year average so fluctuations cannot be determined.

Plantain has a high mineral content, the levels of phosphorous, potassium, sulphur, sodium, calcium, magnesium, boron, cobalt, copper and zinc are similar and above white clover and perennial ryegrass (Wilman and Derrick 1994, Rumball *et al* 1997, Harrington *et al* 2006). There is evidence plantain contains anthelmintic and antimicrobial properties. Plantain contains several biologically active, secondary plant compounds such as mucilage and iridoid glucoside aucubin occur in tissue at levels up to 3% of DM (Stewart 1996) which have shown to influence parasite activity and rumen microbe function (Rumball *et al* 1997). The extent of this effect is not well known, there is only evidence of an effect. There is evidence of a diuretic effect on animals from iridoid compounds contained in plantain, animals had larger kidneys with no effect on renal function resulting in increased water intake and urine flows (Deaker *et al* 1994b, Wilman and Derrick 1994).

Table 2.2.4 Nutritive value of Plantain (*Plantago lanceolata*)

Author	Herbage Quality (% Dry Matter)				
	CP[1]	NDF[2]	ADF[3]	WSC[4]	ME (MJME/kgDM)[5]
<u>New Zealand</u>					
Jackson *et al* 1996	1.75**	23.1	16.6	17.0	-
Burke *et al* 2000 & 2002b	24.7	28.3	-	14.0	-
Harrington *et al* 2006	28.3	-	11.9	-	-
<u>Australia</u>					
Fulkerson *et al*2007					
Autumn	30.0	45.4	29.4	3.5	9.3
Spring	28.1	48.1	34.8	3.6	9.4
Summer	-	-	-	-	-
Winter	24.8	38.3	20.4	14.6	9.9
Hayes *et al* 2010*	17.3	37.3	24.3	-	10.7
USA					
Sanderson et al 2003					
Spring (1998)	18.5	46.7	-	-	-
Summer (1998)	17.9	47.6	-	-	-
Autumn (1998)	14.1	45.1	-	-	-

[1] Crude Protein [2] Neutral Detergent Fibre [3] Acid Detergent Fibre
[4] Water Soluble Carbohydrates [5] Metabolisable Energy
*Cootamundra (Temperate Australia in drought conditions)
** Total N % in Dry Matter

2.2.5 Chicory

The ME value of chicory ranges between 8.7% and 12.5% in literature (Table 2.2.5). The ME value of chicory in New Zealand is limited, Burke *et al* (2000 and 2002b) reported a relatively high ME of

12.5%, similar to clover. In drought conditions the ME remained high at 10.8% (Hayes *et al* 2010) which shows chicory can still produce high quality herbage in dry conditions. The CP content of chicory ranges between 15.2 - 30.7% in literature (Table 2.2.5). The CP content determines the nitrogen content of feed; the CP of chicory is slightly higher than the alternative herb Plantain and therefore the nitrogen content may be higher than Plantain. Lee *et al* (2015) reported a decline in the CP of chicory with an increased grazing interval, the decline was not as rapid or as great as it was in plantain.

The mineral content of chicory is relatively high compared to other grass and legume species. Harrington *et al* (2006) and Hare et al (1987) reported significantly higher concentrations of Phosphorous, Sulphur, Magnesium, Sodium, Copper, Manganese, Zinc and Boron in chicory compared to perennial ryegrass and white clover. Chicory, like plantain, contains concentrations of secondary plant compounds such as sesquiterpene lactones, chicoriin and chicoric acid (Barry 1998). These are part of the plant defensive mechanism against attack from insects (Rees and Harbone 1985) and soil borne microorganisms (Sclerotinia spp of fungi), however high concentrations cause bitterness/aftertaste in milk if it is a sole diet (Rumball 2003b). Chicory cultivars are bred for high concentrations of sesquiterpine lactones to increase persistence ('Puna II') and low concentrations for use in dairy systems ('Choice') (Rumball 2003a,b).

Table 2.2.5 Nutritive value of Chicory (*Cichorium intybus*)

Author	Herbage Quality (% Dry Matter)				
	CP[1]	NDF[2]	ADF[3]	WSC[4]	ME (MJME/kgDM)[5]
New Zealand	-				
Jackson *et al*1996	1.97**	16.8	12.4	11.1	-
Fraser and Rowarth 1996	24.3	17.0	14.6	-	-
Burke *et al* 2000 & 2002b	19.3	23.8	21.2	11.4	12.5
Harrington *et al* 2006	30.7	-	14.7	-	-
Australia					
Fulkerson *et al* 2007					
Autumn	27.8	26.6	24.1	4.0	8.7
Spring	27.3	42.7	22.6	3.2	8.7
Summer	24.9	28.5	19.8	7.7	10.2
Winter	28.9	27.8	27.5	6.2	10.1
Hayes *et al* 2010*	19.9	35.4	21.8	-	10.8
USA					
Sanderson et al 2003					
Spring (1998)	20.0	50.7	-	-	-
Summer (1998)	18.1	43.5	-	-	-
Autumn (1998)	15.2	38.1	-	-	-

[1] Crude Protein [2] Neutral Detergent Fibre [3] Acid Detergent Fibre

[4] Water Soluble Carbohydrates [5] Metabolisable Energy

*Cootamundra (Temperate Australia in drought conditions)

** Total N % in Dry Matter

2.2.6 Lucerne

Lucerne is a high quality feed crop which has an ME value between 9% and 11% (Table 2.2.6), ME values are similar to that of perennial ryegrass, but lower than white clover. The ME was higher in New Zealand research (9.8 - 11%) than Australia (9.0 - 9.8%), suggesting there is a difference in forage quality between environments. The CP levels of lucerne are high and is similar to other legumes such as white clover. The CP ranges of Lucerne are between 20% and 30% in the literature (Table 2.2.6) and white clover is between 15% and 30% (Table 2.2.3). The nitrogen percent (N%) also ranged between 3.3% and 4.0% which is relatively high compared to grass species (perennial ryegrass and cocksfoot) which had an average of 3.1%N (Mills and Moot 2010). The quality of Lucerne (based on CP and ME content) is significantly higher in palatable parts of the plant (upper stems and leaves) than the unpalatable lower stem under irrigation (CP -29% vs 12% and ME – 11.6 vs 7.8MJME/kgDM) (Brown and Moot 2004). The issue with high protein content in forages such as lucerne is very little reaches the small intestine to be absorbed due to rapid degradation in the rumen (Dhiman *et al* 2003)

Table 2.2.6 Nutritive value of Lucerne (*Meticago sativa* L.)

Author	Herbage Quality (% Dry Matter)				
	CP[1]	NDF[2]	ADF[3]	WSC[4]	ME (MJME/kgDM)[5]
<u>New Zealand</u>					
Burke *et al* 2000 & 2002b	29.9	29.5	21.4	8.6	10.9
Burke *et al* 2002a	24.4	32.3	-	12.3	10.0
Harrington *et al* 2006	22.3	36.5	24.7	-	9.8
Mills and Moot 2010					
Year 1 (2007/08)	4.0**	-	-	-	10.9
Year 2 (2008/09)	3.8**	-	-	-	11.0
<u>Australia</u>					
Fulkerson *et al* 2007					
Autumn	-	-	-	-	-
Spring	29.0	32.2	26.3	5.5	9.7
Summer	20.6	35.8	28.3	8.1	9
Winter	30.1	47.2	24.6	5.5	9.3
Hayes *et al* 2010*	22.3	36.5	24.7	-	9.8

[1] Crude Protein [2] Neutral Detergent Fibre [3] Acid Detergent Fibre

[4] Water Soluble Carbohydrates [5] Metabolisable Energy

*Cootamundra (Temperate Australia in drought conditions)

** Total N % in Dry Matter

2.3 Farming and N Losses

Nitrogen losses in grazing pastoral systems are driven largely by urinary N excretion. Grazing ruminants utilise dietary CP in forage, partitioning it into milk and tissue production, metabolic processes, and excreta (dung and urine). Nitrogen is returned to the soil in a small concentrated area as urea-N, some of which is volatised as ammonia, the remaining ammonium is converted into nitrates ($N0_3$) that are available to plants and easily leached from soil (Cameron *et al* 2013). Urination events occur through the loss and replenishment of water in the animal which is a dynamic process controlled by water intake, feed intake and metabolic processes, therefore creating a large amount of variation. The N loading of a urine patch is an important factor for estimating the potential N leached from pasture at a paddock scale, N loading is a function of volume, N concentration and area (Li *et al* 2012). The N load in each urine patch is a function of urine volume, urinary N concentration, and the urine patch surface area which influences the fate of urinary N (Di and Cameron 2002a, Li *et al* 2012, Betteridge *et al* 2013, Ravera *et al* 2015). Large variations in urine volume and N concentration can produce large variations in the N load of urine patches, and consequently can be expected to significantly influence N leaching losses (Li *et al* 2012). Nitrogen loss under urine patches at paddock scale has been reported to be as high as 120kgN/ha/yr, however considering the coverage area the leaching loss reduced to 33kgN/ha/yr (Silva *et al* 1999) (Figure 2.3.1).

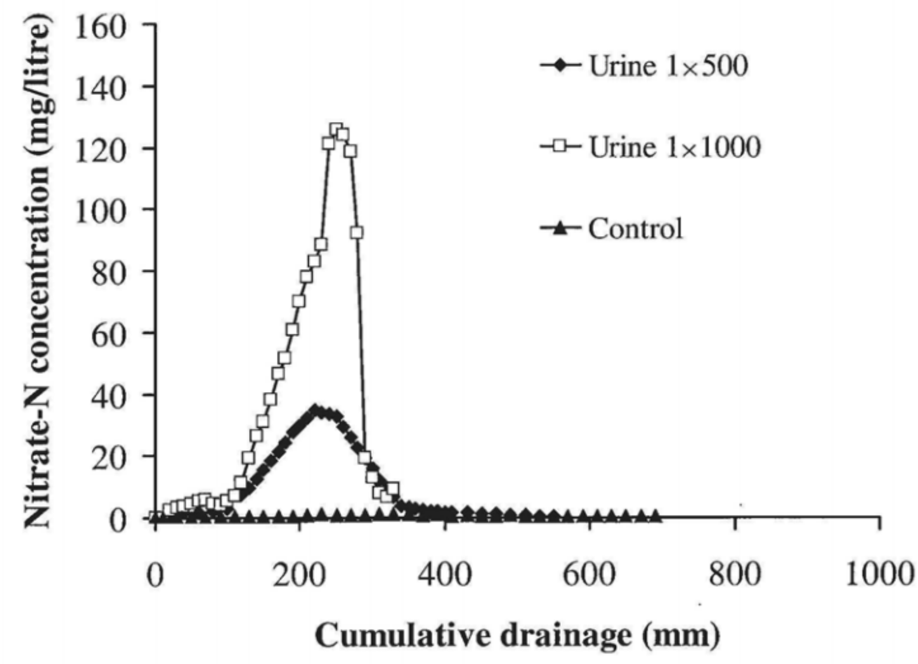

Figure 2.3.1: Concentrations of Nitrate-N leached from different rates of animal urine applied to a pasture soil; Urine 500 = 500kgN/ha/yr and Urine 1000 = 1000kgN/ha/yr. (Source: Cameron and Di 2004, taken from Siva 1999 and Fraser 1994).

Urine Volume

Urine volume affects the wetted area of a urine patch. Urine is regulated in the body by the kidneys, which maintains the body's concentration of electrolytes, acids and bases, minerals and water at adequate levels. To do this the urine concentration and volume are adjusted in response to dietary intake and metabolism changes (Frandson et al., 2006). The volume of urine events in cows is affected by the water intake, this is determined by the concentration of minerals that need to be excreted, if the mineral concentration is high and more needs to be excreted the animal requires more water intake. The concentration of other minerals can influence the urine volume, for example sodium (Na) and potassium (K) regulate osmotic pressure in cells and the diffusion of minerals (in particular N) (Bannink et al., 1999). The level of potassium ingested was has a positive correlation to the CP and N content in dry matter (Betteridge *et al* 1986, Khelil-Arfa *et al* 2012). This was shown in a study by Khelil-Arfa *et al* (2012), the CP content of forage (g/100gDM) and the amount ingested in daily dry matter intake were principle factors affecting urine volume, showing a positive correlation (R^2 = 0.77). This was similar to results found by Holter and Urban (1992) which found a positive correlation (R^2=0.92) in urine output with the dietary CP content included as a factor, cows adjust their water intake to balance total water intake as the CP of the diet changes. These trials also found the dry matter intake (DMI) and dietary dry matter % to be significant factors that affect water intake, and therefore urine volume. For example, at a given DMI level, when the DM content of the feed decreased, the amount of water ingested with feed decreased and the cows had to increase their feed intake (feed water intake) to maintain an amount a total water intake.

The urine volume in dairy cows has considerable variation during the day. In a trial by Betteridge *et al* (1986) investigating the urinary behaviour in steers on pasture found a large variation in urine volume and frequency within and between days. The urine volumes ranged from 0.205L to 3.35L with an average volume of 0.768L. The frequency of urinations per day was highly variable, between 13 and 73 times. The trial reported a significant effect of climate on the variability in urination. In a more recent trial which included dairy cows Betteridge *et al* (2013) reported that urine volume per event ranged between 0.30L and 7.83L, with an average volume of 2.1L. Ravera *et al* (2015) also investigated the variation in urine volume, frequency and concentration in dairy cows grazing kale and fodder beet (5 and 8 cows respectively). The average urine volume was 2.37L, but this varied from 0.5L to 8.6L, the frequency of urination ranged from 3 to 21 per day over both treatments. The results of the last two trials are similar but there is a limitation as the sample sizes are small and it is only done for one year, more data is required to improve the confidence and accuracy of the results.

Urinary N Concentration

The concentration of N in urine affects soil N loading under a urine patch. Temperate dairy systems have very low efficiency of incorporating forage N into milk and tissue growth (Pacheco and Waghorn 2008, Pacheco *et al* 2010), depending on the feed approximately 75-95% of dietary N ingested by ruminants is excreted (Eckard *et al* 2010, Cameron *et al* 2013). This results in highly concentrated urine that can contain between 700-1200kgN/ha in a single urine patch (Haynes and Williams 1993, Di and Cameron 2002a). The inefficiency in the utilisation of dietary N is created by an imbalance between the dietary N intake and the N requirement of the animal, (Pacheco *et al* 2010). New Zealand pastures often contain higher N concentration than required by ruminants, the excess N is not stored in the body and is excreted in urine. Dietary N can limit ruminant production if supply is insufficient relative to animal requirements, the N requirement is expressed as crude protein which indicates the N content of a forage (N=CP/6.25). The CP requirement for grazing animals is 11% for maintenance, 14% for growing cattle and 18% for young or lactating cattle, a CP exceeding 20% are always surplus to requirements (Pacheco and Waghorn, 2008). The urine volume also creates a dilution effect that reduces urine loading in a paddock by reducing urine N concentration and has a greater dispersion (if the increased urine volume results in more urination events) (Costall and Betteridge 2010).

The urine concentration of steers grazing fresh pasture was investigated by Betteridge *et al* (1986), the N concentration in the urine varied between 0.8 and 14.1gN/L. In the more recent study (Betteridge *et al* 2013) the urine concentration of dairy cows grazing pasture ranged between 1.2gN/L to 24.7gN/L with an average concentration of 9.5gN/L. A trial by Bryant *et al* (2014) investigated the effect of WSC levels in pasture on N partitioning, adjusting the WSC concentration through long/short grazing intervals and diurnal fluctuations in WSC (afternoon v's morning). The average urine concentration over all treatments was 4.54gN/L and ranged between 0.9-10.8gN/L, the short regrowth/AM treatment was significantly higher (1.68gN/L) than the long rotation/PM treatment.

Urine N concentration varies diurnally which may be associated with timing of grazing and/or long resting bouts. Betteridge *et al* (1986) found a higher urine concentration at night but measurements were only taken every 6 hours, however this was also found in the later trial in 2013 with higher N loads at night. Relatively large N loads occurred at sunrise when the cows stood up, but thereafter, loads were smaller and urination frequency was higher (Betteridge *et al* 2013). Betteridge *et al* (2013) also found the urinary N loads increased for 10 hours after cows were given a new break of fresh pasture.

Urine patch area

The importance of urine patch area on the N loading to pastures has been identified in previous literature. The urine patch area of cattle has shown a large amount of variation, ranging from 0.16-0.49m^2 (Haynes and Williams 1993). A main source of variation within experiments was the urine volume, but also the slope, wind, soil moisture status (During and Mcnaught 1961, Haynes and Williams 1993), the soil microtopography, vegetation cover, the impact of stock compression and soil macropores (Williams *et al* 1990). Wind affects the spread and variation of urine deposition onto the soil, where slope, micro-topoghraphy and stock compressions affect the flow and spread of urine on the surface. The soil moisture status and macro-porosity of the soil will influence the rate of infiltration, and thus the spread of urine. The measurement technique for patch size was identified as a variation between literature. Ravera *et al* (2015) looked at the actual size of urine patches in dairy cows grazing fodder beet and kale (bare soil), the main factor influencing patch size was the urine volume. The urine patch size increased with an increasing volume. The urine patch area measurements have predominantly defined by the 'wetted' area, this does not take into account the dispersion and spread of the nitrate in the soil. Buckthought *et al* 2016 investigated the 'effective urine patch' by using the uptake of labelled ^{15}N in urine in three zones inside and outside the urine patch to determine the area. The results showed there is lateral movement of urine N in the soil and therefore the effective area can be 3.8 times the size of the urine patch, which can affect the N loading and leaching potential in a urine patch. The overlapping of urine patches is another factor that can affect the N loading at paddock scale, which have the potential to increase N leaching due to a higher N loading, Romera *et al* (2012) reported 8% of an area grazed by dairy cows was covered in multiple urine patches.

2.4 Role of plant species in N loss

Plant species and dietary N

Surplus feed N excreted in cow urine patches are a major form of nitrogen input into the soil to increase leaching (Woodward *et al* 2012). Pastures which include herbs are shown to reduce urine nitrate concentration of dairy cows (Woodward *et* al 2012; Totty *et al* 2013). The results from Woodward *et al* (2012) showed a lower urinary N concentration in cows grazing diverse pastures, 0.26%N (100gN/day) in diverse pastures and 0.62%N (200gN/day) in simple pastures. The reduction in urine N concentration was consistent with results from Edwards *et al* (2015) which showed significant reductions in urine N concentration in diverse pastures.

Urinary N concentration is closely related to the N intake from the feed dry matter and the partitioning of N into milk, urine and faeces. In some studies, cows grazing pastures containing herbs had a significantly lower dietary N intake (at the same DM intake levels) than ryegrass/clover pasture (350gN/cow/day vs 466gN/cow/day), indicating diverse pastures contained less N (Woodward *et al* 2012). Cows grazing diverse pastures also partitioned significantly more N into the milk (79gN/cow/day v's 68gN/cow/day) and the difference in faecal N concentration did not vary significantly, this resulted in a reduced urinary N concentration.

There are some pasture characteristics that have been identified to help reduce the protein utilisation in ruminants to either improve the use in production of milk and meat or to reduce the concentration of N in urine. It has been proposed that grazing forages with elevated levels of condensed tannins and water soluble carbohydrates to lower urinary N concentration (Waghorn et al 2007; Woodward et al 2004). The higher WSC content is thought to improve rumen livestock production by increasing energy availability, synchronising N capture in the rumen and increasing microbial protein availability in the rumen (Bryant *et al* 2009). In the UK a study by Miller *et al* (2001) showed cows in late lactation grazing high sugar ryegrass with elevated levels of WSC had significant reduction in urinary N and an increase in milk N content compared to standard ryegrass, Moorby et al (2006) found similar results in housed dairy cows. The WSC:CP ratio is important for N utilisation and must be >0.7 to show a reduction in urine N (Edwards *et al* 2007), which may require increases of up to 100-200g WSC/kgDM which is a large amount and will be very hard to achieve in New Zealand pastures with high CP content.

Condensed tannins (CT) in pasture species have the potential to improve production in ruminants whilst reducing N losses in the farming system. CT are polyphenolic compounds that occur in a wide range of plants eaten by ruminants and can have a positive and negative effects on digestion and absorption of nutrients, but are not digested themselves (Terrill et al. 1994). An important benefit of CT is the ability to reduce protein degradation in the rumen which allows more undegraded plant protein and amino acids to reach the small intestine, improving protein (N) utilisation. High protein forages are degraded rapidly in the rumen by microbes that produce ammonia which has a poor utilisation, most of which is lost in urine, only 30% of ingested plant material reaches the small intestine (Waghorn *et al* 1998). Forage containing CT concentrations of 5-12% of DM can reduce ammonia concentrations in the rumen by 30-70%. Improving the protein utilisation in the rumen has beneficial effects to production, both in the individual animal and from increased stocking rate (10-30% reduction in feed intake with CT content between 5-12% of DM) (Waghorn *et al* 1998). Selecting species that contain CT have the potential to improve nitrogen utilisation and animal production

Condensed tannins (CT) occur at in several temperate legumes used in New Zealand such as birdsfoot trefoil (*Lotus corniculatus*), Lotus (*Lotus pedunculatus*) and sulla (*Hedysarum coronarium*). Jackson *et al* (1996) analysed the extractable and bound CT and found the probable presence of CT in chicory, plantain and lucerne, and identified concentrations of CT in red clover, perennial ryegrass and summer grass. Plantain was the only forage to contain medium levels of CT (14g/kgDM), however alternative methods showed only traces of CT, the initial levels may have been affected by other plant compounds. The bioactivity of condensed tannins in these forages however is largely unknown.

Secondary iridoid plant compounds (aucubin) have shown evidence of a diuretic effect on grazing animals which may have effects on soil N loading by reducing urine N concentration. Aucubin stimulates both the removal of uric acid from tissues to blood and the excretion of uric acid from the kidneys (Kato, 1946), and is commonly used as a medicinal diuretic. Deaker et al found lambs grazing plantain had larger kidneys than those grazing perennial ryegrass, white clover and chicory, with enhanced kidney function. This may be because of diuretic compounds in plantain making the kidneys work harder and therefore grew larger. The diuretic effect of plantain may reduce the urine N loading in soil by causing animals to have more urination events with smaller volumes of urine to greater disperse the N concentration, therefore reducing soil N loading.

Plant N uptake over winter to reduce N loss

The risk of nitrate leaching is the highest when there is excess mineral N in the soil and there is low plant N uptake and frequent drainage events occurs. Nitrate leaching occurs throughout the year, but is highest between late autumn to early spring. During this period rainfall is typically higher than evapotranspiration rates and soil field capacity, creating drainage events that remove excess nitrates held poorly in the soil (due to negative charge) from the soil. The temperature is also cooler which slows plant growth and therefore the requirement for N in plants, resulting in more nitrates in the soil. A trial by Menneer *et al* 2004 found removing stock from pasture over the winter period can reduce annual nitrate leaching by 60%.

The main factors affecting pasture uptake of N are the amount of N available for uptake and temperature (Goh and Haynes, 1986). The plant N uptake increases with the concentration of plant available N in soil root zones, the uptake of N can exceed plant requirements called 'luxury consumption' (Figure 2.4.1), resulting in pastures having high N concentrations (Whitehead 2000). The temperature affect plant growth, for example most pasture species require a temperature between 5 - 30°C for growth (Whitehead 1995a) and therefore uptake of N, in winter temperatures can be below the requirement for growth and reduce N uptake.

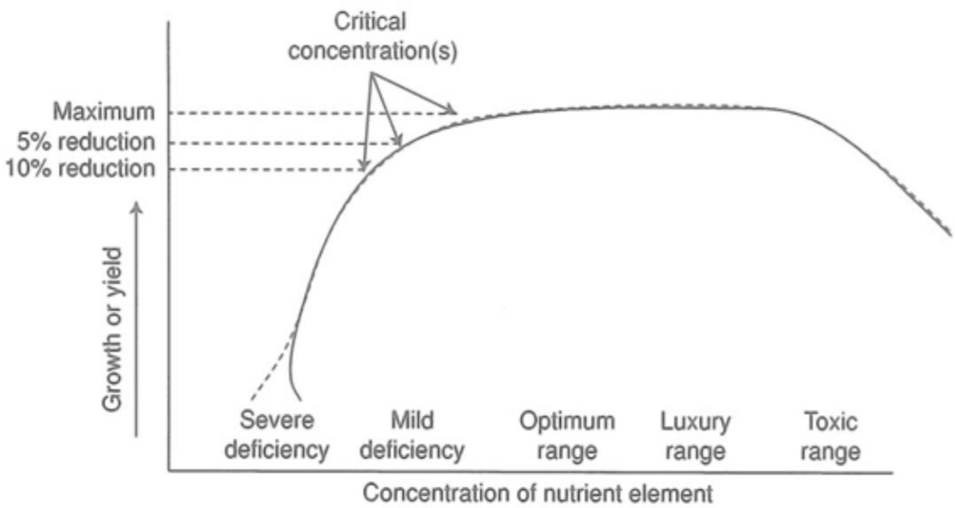

Figure 2.4.1: The percentage of maximum plant growth or yield in relation to the concentration of nutrients in the tissue. (Source Whitehead 2000).

Pasture N uptake is higher under urine patches which is a function of increased plant available nitrogen in the soil, resulting in higher DM yields and N content in pasture. (Haynes and Williams 1993, Fraser *et al* 1994, Clough *et al* 1996, Di *et al* 2002). Literature investigating the recovery of labelled ^{15}N varies between 20% and 55% depending on the rate of urine N applied and the time taken to recover it (Ledgard *et al* 1982, Fraser *et al* 1992,, Clough et al 1996, 1998, Williams *et al* 1998, Di *et al* 2002) Pasture 15N recoveries tended to be lower after autumn or winter urine application due to greater leaching and denitrification losses of N, recovery spring-applied urinary N is generally higher because temperatures suite pasture growth for a longer period of the day compared to winter (Ball & Field 1982). To increase plant N uptake without compromising DM production in winter plant species with high winter growth should be sown, for example Italian Ryegrass. The higher cool season growth would expect to have higher yields with greater N uptake, Moir *et al* 2013 found annual/Italian ryegrass cultivars had increased N uptake and can potentially reduce N leaching under high N loading areas (urine patches).

Chapter 3

Materials and Methods

3.1 Experimental Site and Design

The trial was conducted at the Lincoln University Research Dairy Farm in Canterbury, New Zealand (Longitude 172°28'E; Latitude 43°38'S) between March and September 2016. The site has an annual rainfall of 620mm (40-year average) and is supplemented with irrigation. The soil type in the F1 block is predominantly a Flaxton silty loam which is a poorly drained soil and the F5 and F6 block consists of predominantly a Wakanui silt loam over sand which is imperfectly drained (see Plate 1). Soil tests for the trial areas were taken in August 2015 (shown in Table 3.1.1). Fertiliser and effluent is applied to the blocks annually. In November 2014 Urea was applied to all the F blocks used in the trial at a rate of 50kgN/ha, and was applied again to all the blocks except F2 in April 2015 at a rate of 25kgN/ha. Ammo 31 (NPKS = 30.7-0-0-14.4) was applied at a rate 0f 35kgN/ha to all the blocks in August 2015. Urea was again applied to the F5 and F6 blocks in three applications; September (35kgN/ha), October (25kgN/ha) and November (25kgN/ha). The dairy shed effluent was applied to the F5 and F6 blocks in June.

Table 3.1.1: Soil test results taken in August 2015 (Hill Laboratories) for F1, F2, F5 and F6 blocks.

Block	pH	Olsen P	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Sodium (Na)
	Units	*mg/L*	*me/100g*	*me/100g*	*me/100g*	*me/100g*
F1	6.0	20.0	0.32	7.80	0.80	0.16
F2	6.0	23.0	0.39	7.70	0.88	0.19
F5	6.6	23.0	0.46	12.3	0.93	0.20
F6	6.6	27.0	0.68	11.1	0.96	0.20

The trial consisted of a 2x2 factorial design with two replicates. The replicate was simple versus diverse, with or without Italian ryegrass. The four different pasture treatments were;

1. Control – Perennial Ryegrass and White Clover
2. Italian Pasture – Italian Ryegrass, Perennial Ryegrass and White Clover
3. Diverse Pasture – Perennial Ryegrass, White Clover, Chicory, Plantain and Lucerne mix
4. Diverse + Italian – Italian Ryegrass, Perennial Ryegrass, White Clover, Chicory, Plantain and Lucerne Mix

The sites had previously been drilled with simple and diverse pasture species and established in October 2013. Italian ryegrass was then re-sown into half the paddock (1.5 ha) on February 28, 2015.

Chicory and Plantain were also sown at this time to the diverse pasture at 2kg/ha. The treatments were sown in October 2013 at the following sowing rates;

Table 3.1.2: Sowing rate and sowing date of the pasture species and cultivars.

Species	Common name	Cultivar	Sowing Rate (kg/ha)	
			Oct-13	Feb-15
Lolium perenne	Perennial Ryegrass	Arrow AR1	12	-
Lolium multiflorum	Italian Ryegrass	Asset AR37	-	20
Trifolium repens	White Clover	Weka	3	-
Medicago sativa	Lucerne	Torlesse	8	-
Cichorium intybus	Chicory	Grouse	1.5	2*
Plantago lanceolata	Plantain	Tonic	1.5	2*

* *Sown into Diverse Pasture only (Treatment 3 and 4)*

Plate 3.1.1: This photo illustrates the trial design used on the F1, F2, F5 and F6 blocks of the Lincoln University Research Dairy Farm in Lincoln, New Zealand.

Management

This study was part of a larger experiment which compared milk yield and urine N loss of dairy cows on simple and diverse pastures. Urine patch area was to be measured during the grazing study in March using replicated mobs of Fresian x Jersey dairy cows (n= 12 cows per treatment). The cows had an allowance of 30kgDM/day above ground level, which increased to 35kgDM/day in the second week of the grazing trial. Allocation was determined by measuring pasture mass with a rising plate meter, and the break area was adjusted to provide target allowance. The pre-grazing mass was approximately 3008 ± 102kgDM/ha in the simple pasture and 3251 ± 217kgDM/ha in the diverse pasture; there was some variation during the trial. The cows were milked twice a day at approximately 6.30am and 2.00pm, the cows were given a new break following morning milking each day of the trial. The cows were taken off the property for wintering, the final grazing of the experimental area occurred between the 10th and 30th of April, cows left the farm on the 5th of May and returned to the farm on the 21st of August. The following methodology outlines two parts of this study which in Experiment 1 addresses the first research question: what is the effect of pasture type on urine patch area? Experiment 2 addresses the second research question: are there differences in herbage N uptake between pasture types during winter?

3.2 Experiment Measurements

3.2.1 Experiment 1

Urine volume and frequency

This trial was done in conjunction with another trial using sensor harnesses to measure the volume, frequency and the nitrogen concentration of cow urine for 24 hours a day. The methods regarding the use of sensor harnesses are described by Betteridge *et al* (2013 NZGA). Ten cows in the Simple and Diverse treatments were fitted with urine harnesses for 5 consecutive days to determine urine volume, N% and frequency of urination.

Urine Patch Area

Photos of urination events were taken in both the morning (before the cows were retrieved for morning milking) between 5.00 and 5.45 and in the afternoon (before afternoon milking between 1.00 and 1.45) to capture variation in the urine volume during the day. To determine the size of the cow urine patch a thermal image was taken using a thermal camera attachment and application (FLIR) on an IPhone. Within two minutes of observing a urination event a laminated marker card (Area = 10cm x 10cm) was placed on the patch to provide a scale in the thermal image (Plate 2). At each urination event a GPS waypoint (Garmin etrex 10) was taken and linked to each photo so the patch could later be located. The area of each urine patch was determined using the Sketch and Calc computer programme. Each photo is uploaded into the software and the outside edge of the urine patch is sketched, allowing the programme to determine the area inside the sketch. When drawing around the urine patch photos the significantly bright yellow and pink areas were included (see plate 2), any gaps that were not covered by urine were not included in the urine patch area.

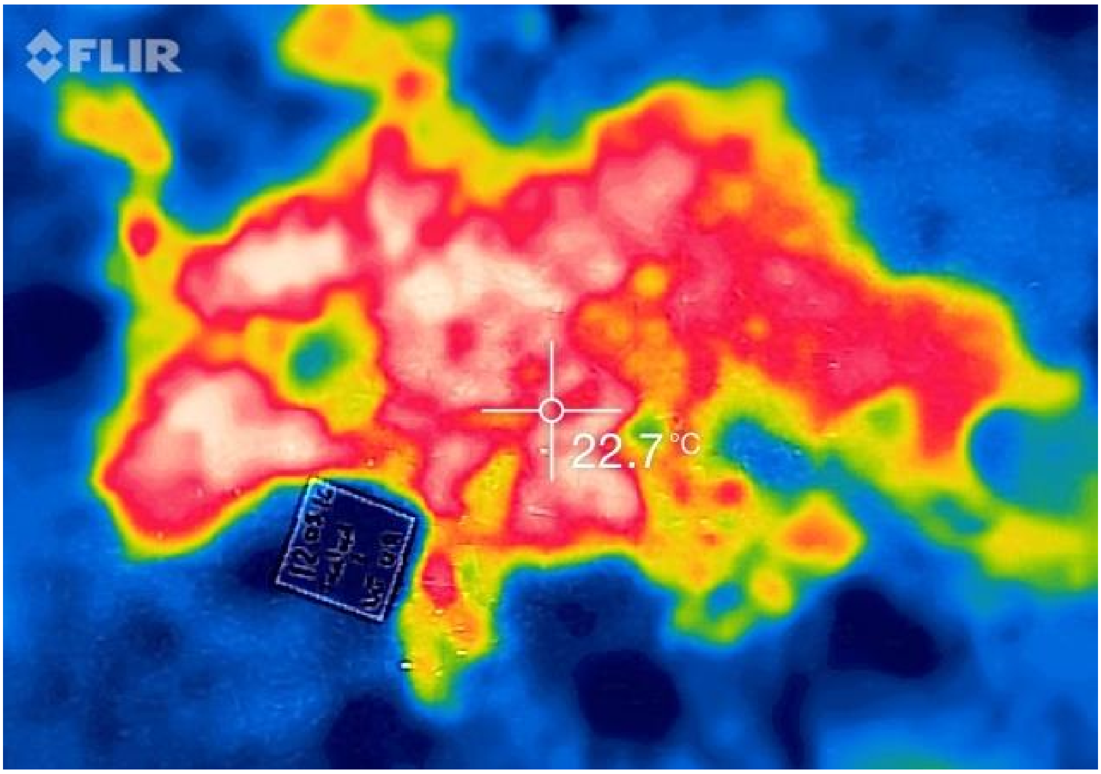

Plate 3.2.1: Thermal image of a urine patch, the area covered by urine is highlighted in bright yellow and pink.

Urine Patch Area Calibration

To estimate soil N loading, urine volume of the urine patch was determined using calibration equations. To describe the relationship between urinary volume and the urine patch area further thermal photos were taken of simulated urine patches to create a calibration curve. Urinary events were simulated by applying known volumes off warm water from the height of a cow's vulva (1.2m) (Ravera *et al* 2015) to each pasture treatment at a range of volumes. There were 10 simulated urine volumes used in the calibration: (i) 0.5L, (ii) 1.0L, (iii) 1.5L, (iv) 2.0L, (v) 2.5L, (vi) 3.0L, (vii) 3.5L, (viii) 4.0L, (xi) 4.5L and (x) 5.0L. Urine patch calibrations were taken for both pre-grazing and post-grazing pasture mass treatments, with each volume replicated 4 times. The areas were calculated using the same computer programme and protocol as the urine patch areas were calculated and plotted against each volume.

3.2.2 Experiment 2

Pasture Growth

A rising plate meter was used to measure the pasture mass accumulation both in a urine patch and outside (Non –urine patch) the urine patches during pasture growth through autumn and winter. Using the GPS waypoints recorded in Experiment 1, four random urine patches from each treatment replicate were monitored. The DM accumulation was measured inside and outside the same urine patches every two weeks from the 15th of April 2016 until it was grazed following the 22nd of July.

Pasture Samples, Botanical Composition and N%

The pasture cuts were collected between the 1st and 12th of August when pastures had reached an average pre-grazing mass of 3419 ±118kg DM/ha in the simple pastures and 3390 ±188kgDM/ha in the diverse pastures. Identification of urine patches was based on GPS readings and visual assessment when distinguishing between the inside the urine patch and outside the urine patch (Non-urine patch). Moir *et al* (2010) assessed urine patches as being areas of lush, dense pasture growth, typical of large pasture nitrogen response and also that the ongoing measurements allowed a more accurate assessment of urine depositions. This trial had relatively accurate (within 1m) GPS waypoints of all the urine patches used in the trial, so there was a visual urine patch at each one. To identify the edge between the urine patch and the area outside the urine patch was assessed by the difference in the pasture density and amount of bare ground shown, also a large indicator was the change in pasture height, leaf size and leaf colour/shine. The pasture outside the urine patch was far sparser with more ground showing, also the pasture leaves were slightly smaller with a lighter shade of green than inside the patch. This visual assessment of urine patches also held when identifying the patches in the mixed pastures, there was only more pasture species to assess.

Pasture cuts were randomly taken from 10 visually identified urine patches and 10 non-urine patch areas in each treatment replicate. A single quadrat cut ($0.1m^2$) was harvested within the urine patch and from outside the patch or non-urine patch (2*10*8 = 160 pasture samples). Quadrats were cut to ground level using electric hand shears and the herbage placed in labelled bags. The harvested samples were mixed, and sub-samples representative of the sward were removed for nutritive value, DM% and botanical composition. The first sub sample was frozen as quickly as possible to stop the breakdown of proteins in the plant material. The samples were freeze dried and ground before the nitrogen content was determined using calibrated near infra-red spectrophotometry (NIRS). The second sub sample for botanical composition samples was separated into the botanical components: (i) Perennial Ryegrass, (ii) Italian Ryegrass, (iii) Clover, (iv) Plantain, (v) Chicory, (vi) Lucerne, (vii) Weeds and (viii) Dead material. The samples were then oven dried for 48 hours at 60®C and weighed.

3.3 Statistical Analysis

The results were tabulated in excel and then the analyses of variance were done in Genstat 16, results were then graphed using excel.

Data to calculate the growth rates through winter were calculated by finding the difference between DM cover at each recording and dividing it by the number of days between measurements (n=14d). The N% was calculated from NIRS results, using the average of Protein dm and Protein dm% and dividing it by 6.25. The total N in an individual sample (for both inside and outside urine patches) was calculated by multiplying the dry matter yield of the urine patch by the nitrogen % of herbage (in the same sample) divided by 100.

- The average Urine Patch N load (gN/event) = *urine N concentration (gN/L) x average volume per event (L)*
- The average Urine N load (kgN/ha) = *(urine load per event (gN/event) x patch area (m^2) x 10*
- The Urine patch coverage (ha) = (*number of urine patch events per day x cow density (cows/ha) x average urine patch area (m^2)) / 10,000*
    - Cow density is based on allowance of 15kgDM/cow = (*pre-grazing mass – target post-grazing mass) / 15.*
- The total N loading (kgN/ha) = urine patch coverage (ha) x urinary N load (kgN/ha).

Chapter 4

Results

4.1 Climate

Rainfall and Temperature

The monthly rainfall, mean and minimum air temperature for the trial period are shown in Figure 4.1. With the exception of May, which had double the long term average rainfall, the rainfall each month from March to September 2016 was lower than the long-term average. The mean air and soil temperature in autumn (March to May) was respectively 13.9 and 12.0 degrees Celsius. The mean air and soil temperature in winter (June to August) was respectively 7.6 and 5.0 degrees Celsius.

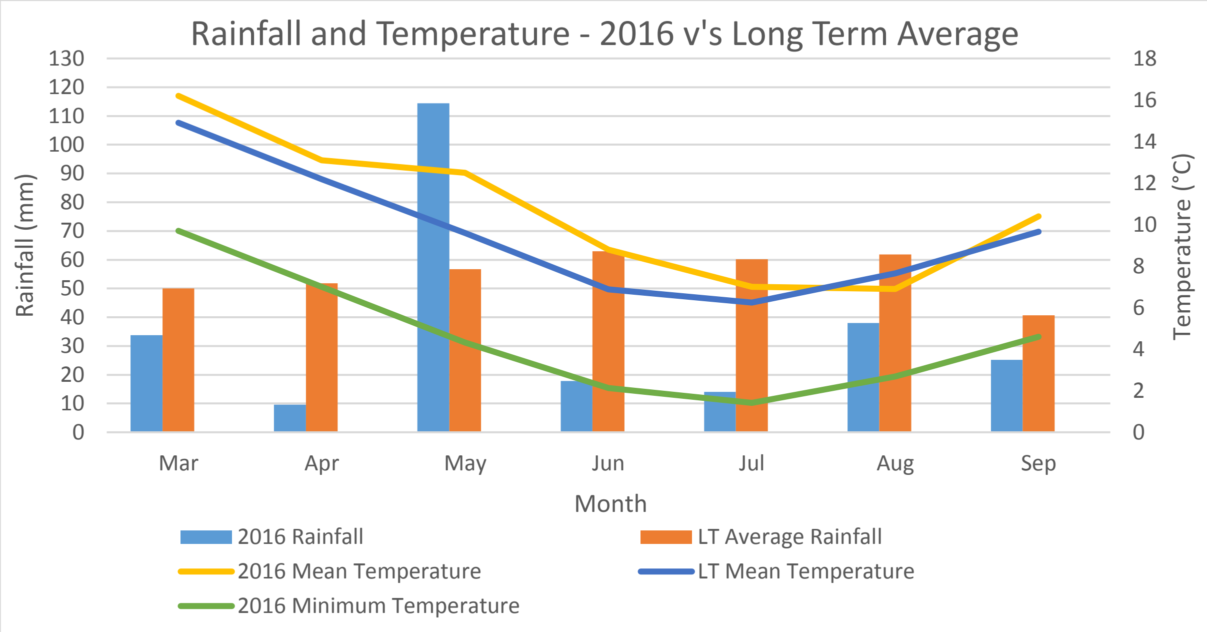

Figure 4.1.1: Average rainfall and Temperature between March and September for 2016 compared to the long-term average (Rainfall since 1976 and Temperature since 1988), and the minimum temperature for 2016. Data is obtained from Lincoln Broadfield's Ews and Els sites (Long 172°47'E Lat 43°628'S) (NIWA, 2016).

4.2 Experiment 1 – Urine Patch Area and Calibration Curves

4.2.1 Urine Patch Area

The average urine patch area and the average maximum and minimum patch areas taken from thermal images of cow urination events in the morning and afternoon are shown in Table 4.2.1. There was no effect of pasture type or Italian ryegrass on urine patch area which ranged from 0.136m^2 to 0.404m^2, with an average urine patch area was 0.253m^2. There was a significant increase in average urine patch area in the morning measurements on short herbage residual compared to the afternoon measurements on high herbage residual (P<0.001).

Table 4.2.1: Average cow urine patch area on pasture treatments; Simple, Simple + Italian, Diverse and Diverse + Italian, obtained in the morning and afternoon during the grazing trial using a thermal imaging camera.

Measurements		Simple Pasture		Diverse Pasture			P Value	
		- Italian	+ Italian	- Italian	+ Italian	SEM	mix*ital*loc	AM/PM
Urine Patch Area	Average	0.243	0.265	0.254	0.251	0.014	NS	-
	AM	0.333	0.355	0.367	0.330	0.203	NS	P<0.001
	PM	0.152	0.175	0.152	0.172			
Maximum Patch Area	Average	0.337	0.376	0.367	0.404	0.300	NS	-
	AM	0.482	0.457	0.510	0.503	0.042	NS	P<0.001
	PM	0.192	0.295	0.223	0.305			
Minimum Patch Area	Average	0.157	0.148	0.151	0.136	0.009	NS	-
	AM	0.204	0.255	0.215	0.194	0.013	NS	P<0.001
	PM	0.110	0.070	0.087	0.079			

4.2.2 Patch Area Calibration

Calibration curves that were generated to quantify and illustrate the relationship between the urine volume and average patch area on short and long pasture treatments; Simple, Simple + Italian, Diverse and Diverse + Italian, are shown in Figure 4.2.1 and Figure 4.2.2. The calibration curves for all treatments on both short and long pasture produced strong polynomial relationships (R^2>0.95 in all treatments on both Short and Long Pastures). There was no effect of pasture mix (Simple or Diverse) and presence of Italian ryegrass on urine patch area in the calibrations (P>0.05).

The difference between the short and long pasture effect on average urine patch area in all four pasture treatments is shown in Figure 4.2.3. The urine patch area at a given volume is significantly higher on short pasture than long pasture at all volumes (P<0.001). The average patch area ranged between 0.104m^2 (0.5L) to 0.429m^2 (5.0L) in short pasture and 0.041m^2 (0.5L) and 0.292m^2 in long pasture. The difference between urine patch area on short and long pasture at a given volume is the smallest at 0.5L (0.0615m^2) and is the largest at 5.0L (0.1367m^2), the difference at 3.0L is also large (0.1358m^2) (Figure 4.2.3).

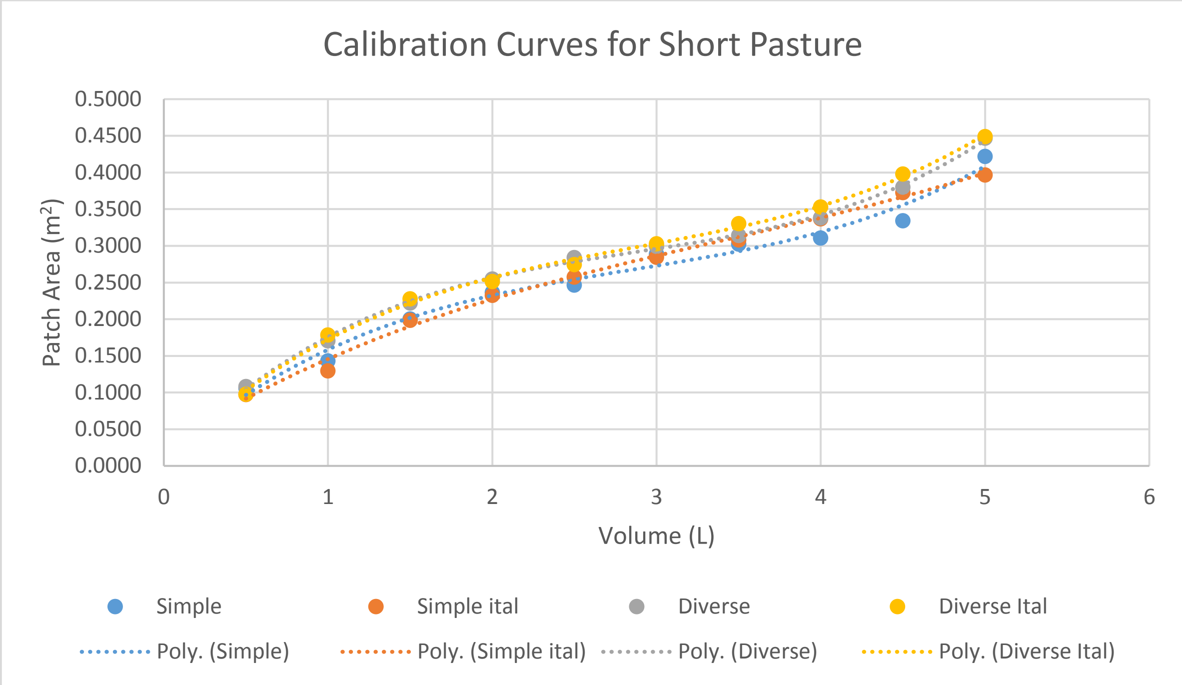

Figure 4.2.1: A calibration curve of the relationship between urine volume and patch area in short (grazed) pasture treatments; Simple (blue), Simple + Italian (red), Diverse (Green) and Diverse + Italian (purple). Curve Equations: Simple $Y=0.0065x^3 - 0.0556x^2 + 0.1954x + 0.012$ ($R^2=0.9813$); Simple + Italian $Y=0.0026x^3 - 0.0268x^2 + 0.1438x + 0.0261$ ($R^2=0.9949$); Diverse $Y=0.0081x^3 - 0.0695x^2 + 0.2325x + 0.0046$ ($R^2=0.9986$); Diverse + Italian $Y=0.007x^3 - 0.061x^2 + 0.218x + 0.0082$ ($R^2=0.9976$).

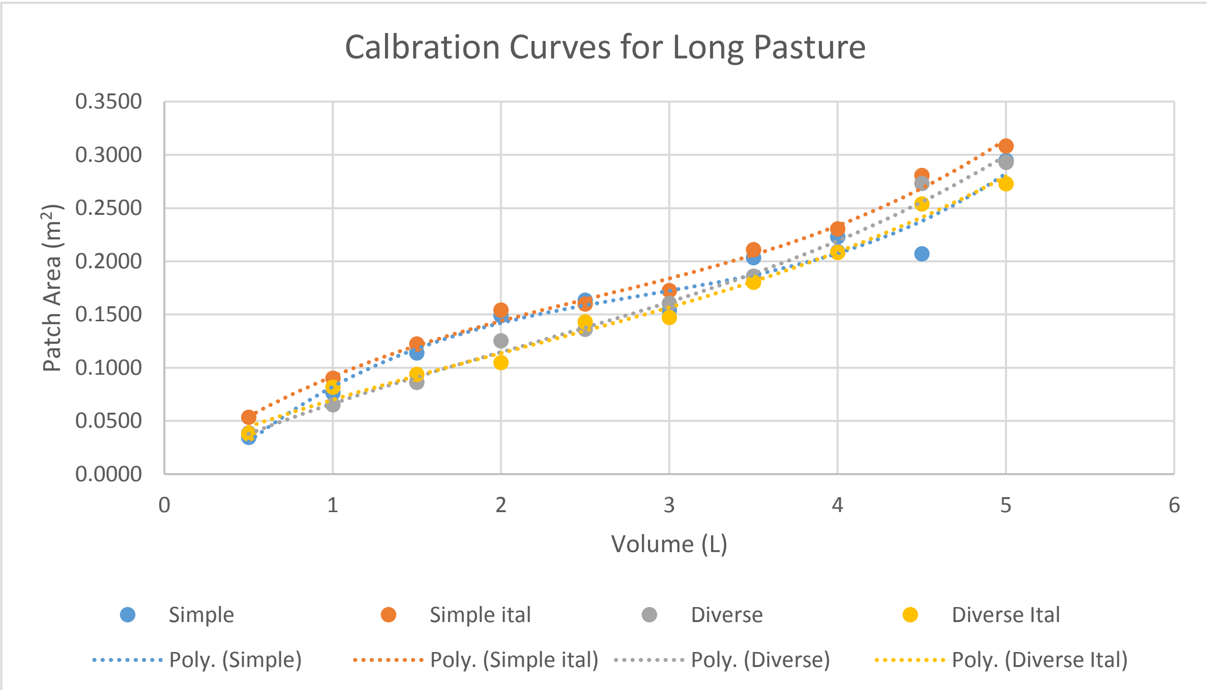

Figure 4.2.2: A calibration curve of the relationship between urine volume and urine patch area in long (pre-graze) pasture treatments; Simple (blue), Simple + Italian (red), Diverse (green) and Diverse + Italian (purple). Curve Equations: Simple $Y=0.0058x^3 - 0.0493x^2 + 0.1676x - 0.0419$ ($R^2=0.9594$); Simple + Italian $Y=0.0036x^3 - 0.028x^2 + 0.1107x + 0.0054$ ($R^2=0.992$); Diverse $Y=0.002x^3 - 0.0127x^2 + 0.0725x + 0.0044$ ($R^2=0.9908$); Diverse + Italian$=0.0016x^3 - 0.0103x^2 + 0.0631x + 0.0153$ ($R^2=0.988$).

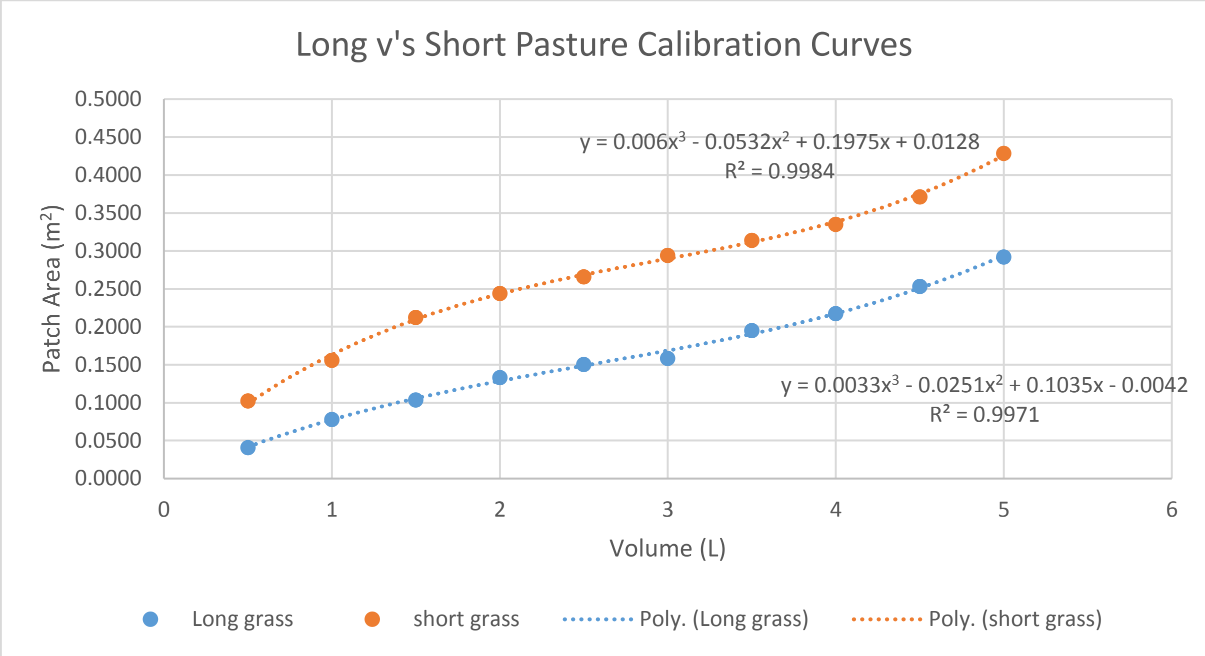

Figure 4.2.3: A comparison between the average calibration curves of all pasture treatments in Long (blue) and Short (red) lengths. Short Pasture R^2=0.9984, Long Pasture R^2=0.9971.

4.3 Experiment 2 – Growth, Botanical Composition and N Uptake

4.3.1 Pasture Growth

The pasture growth rates inside and outside of urine patches in each treatment between April and end of July are shown in Table 4.3.1. There was no effect of diverse mixture or Italian ryegrass on the pasture growth inside and outside of the urine patches (P>0.05). The pasture growth rates in April and June were significantly greater within a urine patch (27.0 and 25. kgDM/ha/day) compared to non-urine patches (17.1 and 4.0kgDM/ha/day).

Table 4.3.1: Pasture growth rates (GR) inside (urine patch) and outside (non-urine patch) of urine patches of each treatment; Simple, Simple + Italian, Diverse and Diverse + Italian, from April to July.

Treatment		April GR (kgDM/ha/day)		May GR (kgDM/ha/day)		June GR (kgDM/ha/day)		July GR (kgDM/ha/day)	
		Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside
Simple	- Italian	26.3	13.4	11.7	6.2	36.2	4.7	5.6	21.4
	+Italian	28.9	16	20.8	9.2	25.6	10.6	28.8	4.9
Diverse	- Italian	26.5	20.1	12.9	9.8	18.3	0	11.5	0
	+Italian	26.1	19.1	11.5	5.6	22.2	4.5	19.1	8.6
SEM		2.16	1.53	3.39	2.4	3.71	2.63	6.42	4.54
P Value	Location	P<0.001		NS		P<0.001		NS	

The dry matter accumulation inside and outside of urine patches on all treatments are presented in Figure 4.3.1 and 4.3.2. The results showed a significant difference in dry matter accumulation between pasture inside and outside of urine patches (P<0.001) during the entire period. The accumulation of DM outside urine patches was not constant, in the last four weeks the growth slowed and remained at similar levels (the Diverse pasture decreased). The DM accumulation becomes more rapid after the 27th May inside urine patches, the accumulation slowed outside the urine patches. Simple pasture mixture showed significantly higher average DM accumulation in the last two measurements, on the 8th and 22nd of July the average accumulated DM in simple treatments was 293±62.7kgDM/ha and 371±155.1kgDM/ha higher than diverse treatments respectively (P= 0.002 and 0.044 respectively). Pasture containing Italian ryegrass had significantly higher DM accumulation on the 8th of June compared to mixes without Italian ryegrass (2727 v's 2558± 62.7kgDM/ha) (P=0.027)

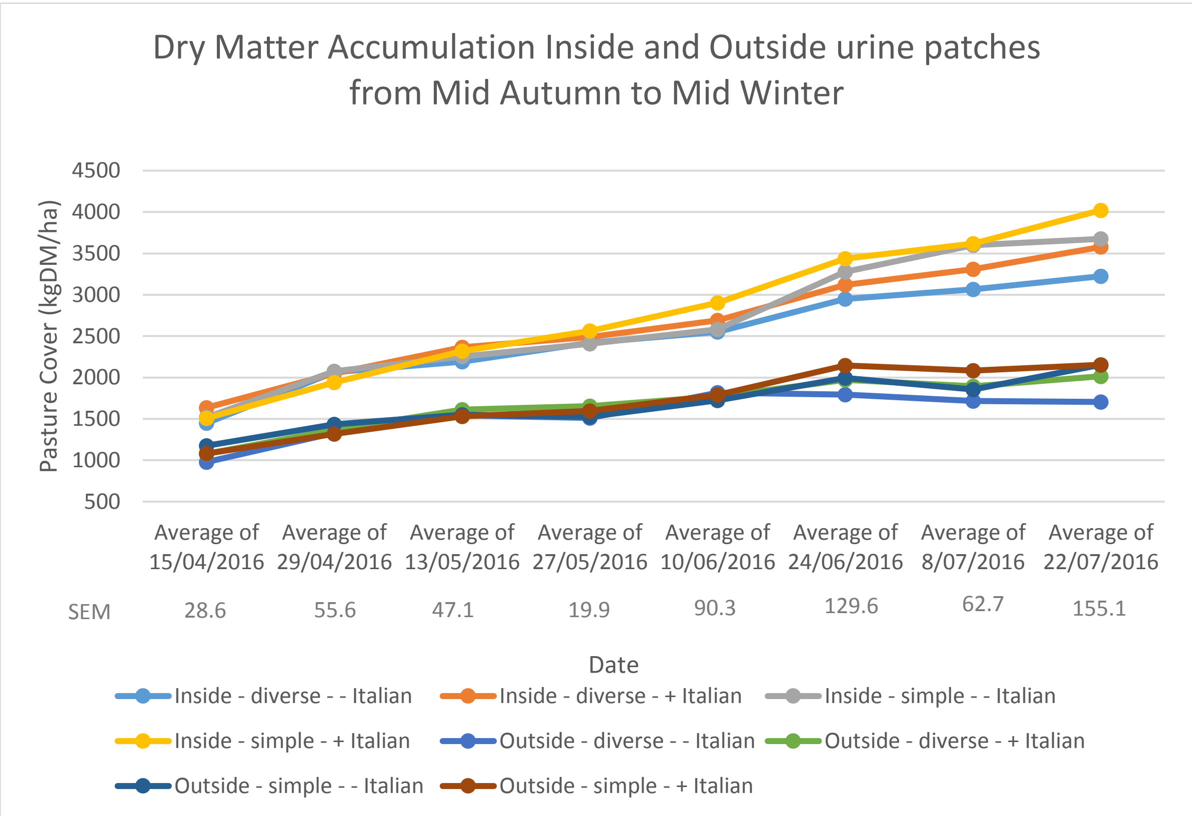

Figure 4.3.1: Dry matter accumulation of pasture treatments; Simple, Simple + Italian, Diverse and Diverse + Italian, from the 15th of April to the 22nd of July from Inside and Outside urine patches.

4.3.2 Botanical Composition

The botanical pasture composition from inside and outside urine patches in each treatment are presented in Figure 4.4.1 and 4.4.2. The average perennial ryegrass content was significantly lower in the diverse pasture treatments (47.5% diverse v's 74.8% simple). The pasture treatments containing italian ryegrass had a significant effect (P<0.01) on the perennial ryegrass content (- Italian 66.7% v's + Italian 55.7%). The perennial ryegrass content was significantly higher outside the urine patches than inside patches in the diverse treatment (52.0% v's 42.9%) (P=0.05) and in the diverse + Italian treatment (72.0% v's 61.4%) (P=0.016). The Italian ryegrass content was higher (P=0.008) outside the urine patches (19%) than inside urine patches (8.5%) for both pasture types. By contrast the clover content was higher (P<0.001) inside urine patches than outside at 4.37% and 1.86% respectively. There was no significant effect of pasture mix or the presence of Italian ryegrass on the content of clover in all treatments and chicory, plantain and Lucerne in the diverse pasture mix. The plantain content in the diverse pastures was higher than chicory ranging between 20-30% and 5-10% respectively, the Lucerne content was very low at <2%. There was no significant interaction between location, mix or use of Italian ryegrass on the weed content (<2%) and dead matter (9.6-17.1%) in all treatments.

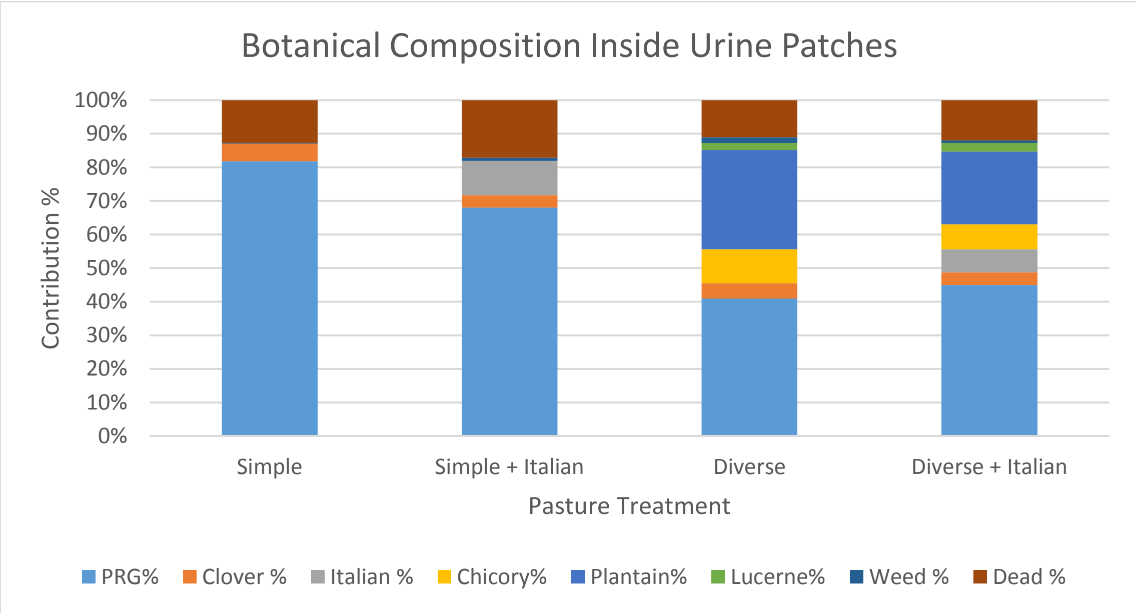

Figure 4.4.1: Pasture composition inside urine patches; percentage of Perennial Ryegrass, White Clover, Italian Ryegrass, Chicory, Plantain, Lucerne, Weeds and Dead Matter.

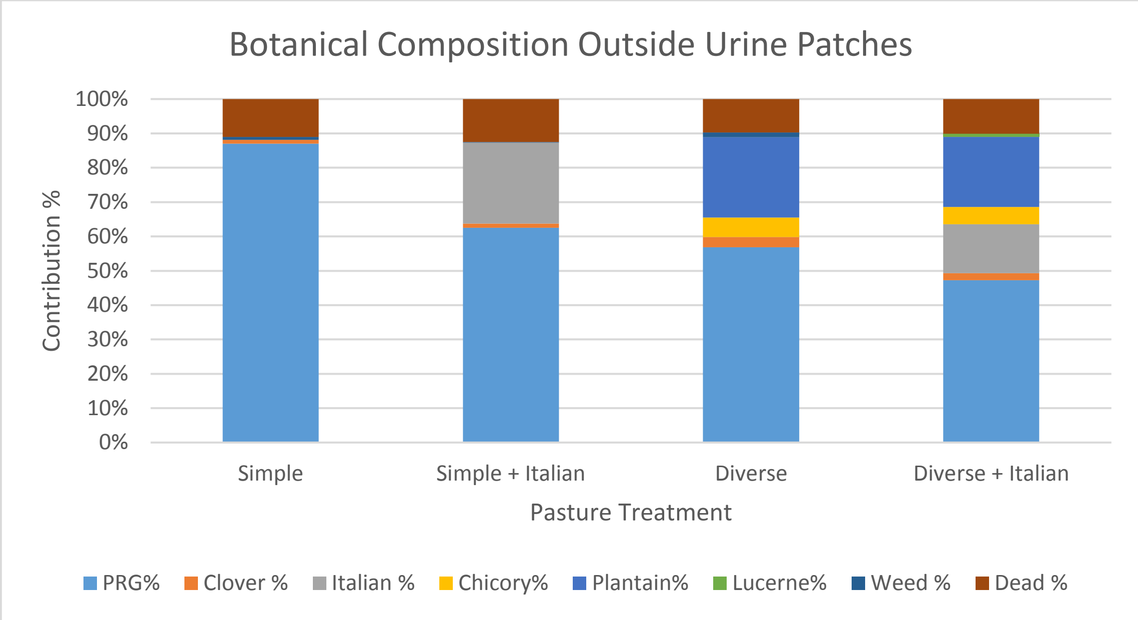

Figure 4.4.2: Pasture composition outside urine patches; % of Perennial Ryegrass, White Clover, Italian Ryegrass, Chicory, Plantain, Lucerne, Weeds and Dead Matter.

4.3.3 Plant N Uptake

The effect of pasture type, Italian ryegrass and urine patch on herbage N uptake is shown Table 4.4.1. The average pasture DM yield inside the urine patches was 4668kgDM/ha, this was higher (P<0.001) than the average DM yield of 2616kgDM/ha outside the urine patches, ranging between 2416 - 4714kgDM/ha. There were no effects of pasture type of italian ryegrass on herbage mass, herbage N% or plant N uptake. There was a significant interaction (P=0.036) between location, mix and presence of Italian on N%, the simple + italian treatment has a larger difference in the N% of pasture inside and outside of urine patches than the diverse +Italian treatment (simple 0.43% v's diverse 0.98%). The N% of the pasture was significantly higher (P<0.001) inside urine patches than outside, the average N% ranged between 2.20% and 3.19%. The average total pasture N uptake inside urine patches was over twice the amount in the pasture outside urine patches (143 v's 62kgN/ha respectively) (P<0.001).

Table 4.4.1: Pasture yield, Nitrogen % and Total N taken from Inside and Outside urine patches in four treatments: Simple, Simple + Italian, Diverse and Diverse + Italian.

Measurements	Location	Simple Pasture		Diverse Pasture			P Value	
		- Italian	+ Italian	- Italian	+ Italian	SEM	Mix*Ital*Loc	Location
Pasture Mass (kgDM/ha)	Urine Patch	4647	4714	4576	4709	168.7	NS	P<0.001
	Non Patch	2750	2849	2416	2448			
N concentration (%)	Urine Patch	3.05	3.19	2.96	3.04	0.066	0.036	P<0.001
	Non Patch	2.36	2.20	2.35	2.60			
N Uptake (kgDM/ha)	Urine Patch	141.3	151.0	135.7	143.1	5.7	NS	P<0.001
	Non Patch	65.1	62.4	57.2	63.6			

4.3.4 Urinary N loading

Using data from the trial and the cow harness sensor trial (Welton *et al* unpublished, Table 5.4.1) the urinary N loading and excess N can be calculated, shown in Table 4.4.2. The results show the N load per event (kgN/ha) was highest in the Simple treatment (631 kgN/ha) and was the lowest in the diverse treatment (547 kgN/ha). The pre-grazing mass was the highest in the Diverse + Italian treatment and was the lowest in the Simple + Italian treatment with a cover of 3356 and 2739kgDM/ha respectively, resulting in a stocking density of 83 and 124 cows per ha in each treatment (Table 4.4.4). Assuming no overlap of urine events the urine coverage area (ha) ranged between 0.0286 and 0.0411ha and was the lowest in the simple + Italian treatment and the highest in the Diverse + Italian treatment. The total N loading was the highest under the simple mixture (23.1 kg N/ha) and was the lowest under the simple + italian mixture (16.5 kg N/ha). The plant uptake of N on the covered area (kg N/ha) ranged between 4.31kgN/ha (Simple + Italian) and 5.88kgN/ha. The excess urinary N was 17.9, 12.2, 15.4 and 16.9kgN/ha in the simple, simple + Italian, Diverse and Diverse + Italian treatments respectively.

Table 4.4.2. The calculated average nitrogen loading and urine patch coverage at patch and paddock scale. Results obtained from data in trial and results found in the sensor harness grazing trial (Table 5.4.1) (Welton et al unpublished).

Measurements	Simple Pasture		Diverse Pasture	
	- Italian	+ Italian	- Italian	+ Italian
Urine Volume (L/event)	2.39	2.39	2.30	2.30
Number of events/day	13.0	13.0	13.2	13.2
Urine N concentration (g N/L)	6.4	6.4	6.0	6.0
N load (g N/event)	15.3	15.3	13.9	13.9
Patch Area (m^2)	0.243	0.265	0.254	0.251
N Load from urine event (kg N/ha)	631	578	547	553
Cow density (cows/ha)	116	83	112	124
Urine Coverage (ha)	0.0366	0.0286	0.0376	0.0411
N loading from urine/ha	23.1	16.5	20.5	22.7
Plant N uptake (kg N/ha)	141.3	151.0	135.7	143.1
Plant uptake (covered area) (kgN/ha)	5.17	4.31	5.10	5.88
Excess N (kgN/ha)	17.9	12.2	15.4	16.9

Chapter 5

Discussion

The purpose of this study was to investigate the effect of diverse pastures and the use of Italian ryegrass on the size of cow urine patch areas and N uptake by plants over winter, as a tool for analysing the total N loading onto soil. The results showed no significant effects of diverse pasture of italian ryegrass on the urine patch size and plant N uptake. There was a significant difference in the size of urine patches in the morning compared to afternoon, and the plant N uptake was significantly higher under urine patches.

5.1 Urine Patch Area

The average cow urine patch size over all the pasture treatments was 0.253m^2. The size of urine patches varied considerably, ranging between 0.070m^2 and 0.51m^2 (Table 4.2.1). The average urine patch size was smaller than the average found by Moir *et al* (2011) of 0.37m^2±, and the range of urine patch areas is much larger than the results from the same trial (0.34 -0.40m^2) on pasture grazed by dairy cows. The main difference in the results is the method used to measure the patch area, Moir *et al* (2011) established the urine patch area from the difference in pasture growth (lushness, density and colour) by visual observation which may occur weeks after the event occurred, it does not measure the wetted area. The range of urine patch area found in the trial is similar to the variation collaborated by Haynes and Williams (1993) which showed dairy cow urine patches ranging between 0.16-0.49m^2. Ravera *et al* (2015) found the average urine patch area of dairy cows grazing forage crops (fodder beet and kale) was between 0.19 and 0.24m^2, however this was directly onto bare soil as it was not covered by pasture which will create variation between results.

In previous literature, the urine patch area was measured using different methods compared with the method used in this study. Ravera *et al* (2015) measured urine patch size by spray painting around the visible edge of the wetted area then calculating area from a photograph of the inside of the painted area. Moir *et al* (2012) measured the area of lush growth, Williams et al (1990) measured the urine patch area using a bromide solution and Buckthought *et al* (2016) that used labelled ^{15}N to distinguish effective area. There is no literature using thermal imaging, so no comparisons can be made to the reliability of the method used. The method is reliant on the accuracy of the Sketch and Calc software to estimate the area and may have human variation whilst drawing around each urine patch.

The calibration curves generated show the pasture height (pre-grazing v's post-grazing) has a significant effect on the urine patch area at different volumes (Figure 4.2.3). Using the calibration equation and average urine patch area results for am and pm, the average predicted morning urine volume was 4.8 litres compared to 0.75 litres in the afternoon. The urine harness results found the average urine volume in am and pm was approximately 3.0 and 2.1 L respectively. The calibration curve results therefore agree that morning volumes were larger than in the afternoon. The differences between predicted and actual urine volume may be due to calibration taking place in a different season (August) and at a different time of day (between 10.00am and 4.00pm) compared to when the urine patch data was collected (March, at approximately 5.30am and 1.30pm). The range in estimated urine volumes calculated from the calibration curve (between 0.75L (am) and 4.8L (pm)) may differ from what was found in the sensor trial, but they are not unrealistic. Betteridge *et al* (2013) and Ravera *et al* (2013) reported urine volumes ranging between 0.30-7.83L and 0.5-8.6L respectfully.

There was no effect of pasture diversity on the urine patch area in Autumn. It was hypothesised that diverse pastures containing herbs such as plantain may have diuretic effects and increase urine volume (O'Connell *et al* 2016) and therefore increase patch area. A trial by O'connell *et al* (2016) found plantain had a diuretic effect on sheep, the urine volume of sheep on plantain was significantly higher on the first day (1.5L) and remained 0.5L higher than sheep grazing perennial ryegrass/whiteclover pasture. There is a high possibility this effect can occur in dairy cows grazing plantain, a larger urine volume has shown to increase the size of urine patches. The evidence in the trial (O'connell *et al* 2016) measured the total daily urine volume, there was no results showing the size of urination events which may have been smaller and more frequent which would not increase urine patch area. The urine volumes measured in the harness trial did not show any significant difference which may explain why there was no effect of diverse pastures on the urine patch area.

There was a significant difference in the urine patch area's depending on the time the urine patches were photographed ($P<0.001$). The urine patches photographed before morning milking were on average 2.12 times larger than the area of urine patches measured before the afternoon milking. Urinary N loading is a factor of urine volume, N concentration and deposition area (Romera 2012). The results found would imply the larger urine patches in the morning will reduce the total N load/ha on the covered area, not the N load per urination event. Therefore, if cows are to be removed from pasture it should be in the afternoon to reduce N loading, but this may have implications on stock performance.

A factor that can influence urine patch area is the volume of urine which has shown to affect the urine patch area (calibration curves). In previous literature, it is assumed the urine volume is higher in the morning as a cause of higher urine N loading (Betteridge *et al* 1986, 2013). Ravera *et al* (2015) found afternoon urine volume between 1-4pm was lower than the daily average. Larger urine volumes result in a higher potential for pooled water to overcome unevenness on the surface and spread over a larger surface area. The volume of urine can in part explain the large variation in urine patch area. The large diurnal and between cow variation in urine volumes is well documented in previous literature (Betteridge *et al* 1986 and 2013, Li *et al* 2012, Ravera *et al* 2015).

The second factor that will affect the urine patch area at different measurement times is the pasture cover. The cows in the trial received a new break after morning milking, therefore the pasture cover was significantly higher before milking in the afternoon than the morning. Cows had approximately four hours of grazing before the afternoon measurements compared to approximately 18 hours of time to graze before the morning urine patches were photographed. The effect of pasture cover is illustrated in the calibration curves which were done on short pasture (post-grazing) and long pasture (pre-grazing) which shows urine patches in short pasture were approximately % larger than those in the afternoon at a given volume. The pasture cover in the afternoon was not recorded when photographing urine patches during the grazing trial due to limited time and people to help, however the effect can be assumed to occur but will be lower than the calibration curve difference as the pasture has been grazed. A higher pasture cover may have buffered the initial impact of urine flow and therefore slowed the potential for urine spread on the soil surface, also the leaves and stolon density was thicker which will limit the pathways of spread.

Other factors were noted to affected the urine patch area during the urine patch measurements and calibrations. The paddock microtopography and unevenness due to cattle impact during grazing affected the spread of urine by creating areas where urine pooled or created a preferential flow over the surface. The wind was a significant factor that could not be controlled during urine patch area measurements, it affected where the urine landed on the surface and the potential of urine flow, therefore affecting the surface spread and created more irregular urine patches. The wind factor is important as it occurs in all outdoor faming systems, especially Canterbury, in this trial the wind factor is somewhat considered, however it may have increased variation and error when drawing the urine area. These factors have also been noted in previous literature, along with the soil moisture status and surface macro-porosity, as factors effecting the spread of urine (During and Mcnaught 1961, Williams *et al* 1990, Haynes and Williams 1993). The effect of these factors on urine patch area is hard to measure and quantify, but will contribute to a greater variation in patch area.

5.2 Nitrogen Uptake

The average pasture N uptake over the period from May to August was over two-fold higher inside urine patches compared to outside urine patches, 143 v's 62 kgN/ha respectively (Table 4.4.1). The effect of urine was also found by Silva (1999) who reported a two fold increase in N uptake within urine patches. The N uptake was calculated from the DM yield and the N concentration in the pasture. There was also a significant difference between DM yield and pasture N % inside and outside of urine patches. the increase in these factors results in a significantly higher total pasture N (N uptake). There was no effect of mixture (Diverse v's Simple) or the presence of Italian ryegrass in DM yield and N uptake between pasture treatments.

The pasture yield and N% of a pasture is a function of increased plant available N in the soil which is well documented (Haynes and Williams 1993, Fraser *et al* 1994, Clough *et al* 1996, Di *et al* 2002), hence under the urine patch the growth was significantly higher. The yield is increased by approximately 2000kgDM/ha inside urine patches compared to outside urine patches, which is commonly known and is visually obvious. The dry matter accumulation inside urine patches was significantly higher inside urine patches (Figure 4.3.1). The climate is a factor affecting N uptake (Goh and Haynes 1986) through plant growth. The temperature was warmer than usual, supporting high growth throughout winter as temperatures (both soil and air) were sufficient for plant growth and N uptake (Whitehead 1995a). Italian ryegrass was used as it has shown potential to increase growth and N uptake over winter (Moir *et al* 2013, Malcolm *et al* 2014). The effect was not shown in this trial which is probably due to the very low Italian ryegrass content as a result of the high perennial ryegrass content dominating pastures (average 61%), studies have shown perennial ryegrass is likely to dominate when the mineral N input was high (Haynes 1981; Ledgard et al. 1987).

The average N% in the pastures was 2.7% and was 28% higher inside urine patches than outside urine patches. The N concentration is influenced by the plant available N in the soil which is higher inside urine patches. Legumes such as white clover and Lucerne have high CP content, ranging upward of 20-30% (Table 2.2.3) and N concentrations as high as 4% (Rattray 1974, Mills and Moot 2010). The legume content in the pasture is very low, averaging only 3-4%, thus it is not likely to significantly affect the N%. Legume content varies but is usually between 15-40% in standard mixes and as high as 52% in diverse mixes (Daly *et al* 1996). The clover was slightly higher inside urine patches which is surprising as clover is sensitive to competition for sunlight which will occur more under a urine patch and doesn't respond as well as other grass and herb species to N (Andrews *et al* 2007, Woodfield and Caradus 1996, Caradus *et al* 1996).

There are limitations to the methods used for N uptake. The N taken up by pasture may not be urinary N only, it may have come from mineral N existing in the soil and fixed from the atmosphere which cannot be determined with the data retrieved from the trial. It is therefore assumed that the plant N uptake is reducing the pool of mineral N, most of which occurred from urine deposition. During the pasture growth measurements, the results started to increase in variability, shown by the standard error increases from July (Table 4.3.1 and Figure 4.3.1). This may explain variable results as the pasture height increased it started to fall over (lodge), reducing the accuracy of the rising plate meter recording (Powell *et al* 2012). The range of species in a mix is likely to alter the accuracy of the measurements due to the differing bulk densities eg. Some have greater bulk density at the top (dicots) and some at the base (monocots).

5.3 N loading at farm scale

The average N loading per event ranged between 547 and 631kgN/ha (Table 4.4.2), which fits within the range between 500-1000kgN/ha by dairy cows reported by Cameron (1992). The average total N load/ha in each treatment was very similar between all treatments, ranging between 16.5 and 23.1kgN/ha (Table 4.4.2). The total N loading per ha found in the calculation is lower than that found by Silva (1999), reporting an N loading of 33kgN/ha in a grazed pasture system. The difference in the N loading results may be due to the difference in methods, Silva (1999) estimated N loading using lysimeter treatments which estimated paddock coverage as a % from past research, where the estimate made on this trial is done from actual data on urine volume, N concentration and urine patch sizes found. The urine patch area and the cow density were the main factors to affect the total N loading in these calculations.

The factors that affected the total N load is urine coverage and the N load per urine patch. The N loading in an individual patch is a function of urine volume, N% and patch area. There was no significant difference in the urine volume, N% and urine patch area between all the treatments. The N load in urine patches was very similar between the treatments, the smaller urine patch area in the simple - italian treatment resulted in a higher N loading/event. Increasing the urine patch area will dilute the N loading per event (Lee *et al* 2012) as the concentration is spread over a larger area. The results from the trial found the difference in urine patch areas was not large enough to significantly affect the total N loading/ha.

A second factor affecting the overall N load was the urine coverage. There was no effect of pasture treatment on the frequency of urination so urine coverage thus becomes very sensitive to cow numbers. Cow density was based on an pre-grazing pasture mass and animal allowance. The average cow density was higher on the diverse mixtures (99.5 vs 118 cows/ha). The presence of herbs possibly increased pre-grazing pasture cover (Nobilly *et al* 2013), however it is unlikely. The main difference occurs in the simple + italian treatment which had a considerably lower pasture mass (Table 5.4.2). The low italian ryegrass content in the pasture may have contributed little to the pasture cover. Italian ryegrass has good cool season growth (de Ruiter 2007), the trial was done in April when the temperature was the warmest so the full effect of italian ryegrass on the pasture cover may not be shown. This is illustrated in Figure 4.3.1, treatments containing Italian ryegrass had significantly higher DM accumulation in the later stages of measurement.

This calculation is a general trend showing the how the factors can affect the overall urine N loading, the results have limited accuracy. The calculated urine N loading is only based on the average data found, it doesn't account for variations in urine volume, N concentration and patch area (also affected by pasture height). The variation of factors affecting the N loading onto a pasture variation important and can create over and under estimations of N leaching between 5-8% (Lee et al 2012). At farm scale a considerable amount of urine is excreted on stock raceways and in milking sheds which will reduce the urine coverage and N loading in a farming system (Haynes and Williams 1993). The research does not take into account overlapping urine patches. Romera et al. (2012) determined that 8% of a pasture paddock area grazed by dairy cattle was covered in multiple urine patches where 39% of the total urine volume was deposited on overlapping patch areas. Ammonia volatilisation from urine patches is a factor that reduces the overall N loading and should be acknowledged, however this could not be analysed in the trial.

Pasture yield and N uptake was not significantly different between treatments (Table 4.4.1) and the N uptake over the covered area was therefore similar, ranging between 4.31 and 5.88kgN/ha. If the N contained in pasture after growth over autumn/winter is assumed to come from urine patch, the total N loading from urine/ha is reduced by 22-26%. The excess N is therefore very similar between treatments, ranging between 12.2 and 17.9kgN/ha. To reduce N leaching by increasing the pasture growth in winter to increase the uptake of N may be of benefit. However increasing pasture growth raises the cow density on the pasture before winter, the increase in N loading from more cows outweighed the benefits of increased pasture growth, mainly due to the insignificant increase in yield and N uptake in treatments containing Italian ryegrass.

Conclusions

The urine patch area is an important factor in the estimation of N leaching from a farming system, along with urine volume and N concentration. Neither increasing pasture diversity nor adding Italian ryegrass altered variables driving N loading from urine patches. This is likely to have been due to low proportions of these species in the pasture mix, further research is required on species proportions to elucidate the effects of specific plants on N loading. The diurnal variation in urine volume and changes in pasture cover over the length of time grazed times become very important in the size of urine patch areas. This can affect the time of day animals are given a new break in order to coincide low pasture mass with higher volumes to dilute the N loading onto pasture. In future, a trial should look at the diurnal variation in urine patch size which seems to change at different times of the day due to pasture cover and urine volume variation. This trial only looked at the urine patch area at two times of the day, if the patch areas were recorded throughout the day it will give a more accurate representation of urine patch area changes, enabling the total N loading of a 24hr grazing to be estimated, reducing the error of calibrations which don't necessarily take into account the daily variation. Providing this information will be useful in programmes that estimate N loss from farming systems eg Overseer.

Appendices

5.4 Cow Information in Grazing Sensor Trial

Table 5.4.1: The average cow urination volume, frequency, urinary N concentration and stocking rate on the simple and diverse pasture treatments in the grazing trial in March (Source – Canterbury grazing Trial, Welton et al unpublished).

Measurements	Simple Pasture		Diverse Pasture		
	- Italian	+ Italian	- Italian	+ Italian	P Value
Urinary Volume (L/cow/day)	29.0	-	28.0	-	NS
Event volume (L/event)	2.30	-	2.39	-	NS
Time Between Events (hrs)	0.076	-	0.077	-	NS
Urine Frequency (events/day)	13.0	-	13.2	-	NS
Urinary N Concentration (gN/L)	6.4	-	6.0	-	NS

Table 5.4.2. The pre-grazing mass of each treatment and the cow density (cows/ha), based on an allowance of 15kgDM/cow/day in each treatment.

	Simple Pasture		Diverse Pasture	
Cow Density Calculation	- Italian	+ Italian	- Italian	+ Italian
Average pre-grazing mass (kgDM/ha)	3233	2739	3178	3356
Target post-grazing mass (kgDM/ha)	1500	1500	1500	1500
Stocking density (cows/ha)-15kg allowance	116	83	112	124

References

Andrews, M.; Scholefield, D.; Abberton, M.T.; Mckenzie, B.A.; Hodge, S.; Raven, J.A. (2007). Use of white clover as an alternative to nitrogen fertiliser for dairy pastures in nitrate vulnerable zones in the UK: productivity, environmental impact and economic considerations. Annals of Applied Biology, 151: 11-23

Ball, P.R.; Molloy, L.F.; Ross, D.J. (1978). Influence of fertiliser nitrogen on herbage dry matter and nitrogen yields and botanical composition of a grassed grass/clover pasture. New Zealand Journal of Agricultural Research 21: 47–55.

Ball, R.P. and Field, T.R.O. (1982). Responses to Nitrogen as affected by pasture characteristics, season and grazing management. *In*: Lynch, P.B e.d. Nitrogen Fertiliser in New Zealand Agriculture. Pp 45-64

Bannink, A.; Valk, H.; and Van Vuuren A.M. (1999). Intake and excretion of sodium, potassium, and nitrogen and the effects on urine production by lactating dairy cows. Journal of Dairy Science 82, 1008–1018.

Barrell, L.G.; Burke, J.L.; Attwood, G.T.; Brookes, I.M.; Waghorn, G.C. (2000). Preparation of fresh forages for incubation and prediction of nutritive value. Proceedings of the New Zealand Society of Animal Production 60:

Barry, T,N. (1998). The feeding value of chicory (Cichorium intybus) for ruminant livestock. Journal of Agricultural Science, Cambridge, 131: 251–257

Bennett, S.M. (2012). Dry matter production of Lucerne (*Meticago sativa* L.) at Ashley Denne. B. Agrl Sci Hons Dissertation. Lincoln University.

Betteridge, K.; Andrewes, W. G. K.; & Sedcole, J. R. (1986). Intake and excretion of nitrogen, potassium and phosphorus by grazing steers. Journal of Agricultural Science, 106(2), 393-404.

Betteridge, K.; Costall, D. A.; Li, F. Y.; 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.

Brock, J.L. (1973). Growth and nitrogen fixation of pure stands of three pasture legumes with high/low phosphate. New Zealand Journal of Agricultural Research 16: 483491.

Brock, J.L.; Caradus, J.R.; Hay, M.J.M. (1989). Fifty years of white clover research in New Zealand. Proceedings of the New Zealand Grassland Association 50: 2539.

Brougham, R.W. (1960). The effect of frequent hard grazings at different times of the year on the productivity and species yields of a grass-clover pasture. New Zealand Journal of Agricultural Research 3: 125-136.

Brown, H.E.; Moot, D.J.; Pollock, K.M.; Inch, C. (2000). Dry matter production of irrigated chicory, lucerne and red clover in Canterbury. Proceedings of the New Zealand Agronomy Society 30: 129-137

Brown, H.E.; Moot, D.J.; Pollock, K.M. (2003). Long term growth rates and water extraction patterns of dryland Chicory, Lucerne and Red clover. *In* Legumes for dryland pasture, proceedings of the Grasslands Symposium. November, pp 91-95.

Brown, H.E.; Moot, D.J. (2004). Quality and quantity of chicory, Lucerne and red clover production under irrigation. Proceedings of the New Zealand Grassland Association 66, 257–264.

Brown, H.E.; Moot, D.J.; Pollock, K. 2005. Herbage production, persistence, nutritive characteristics and water use of perennial forages grown over 6 years on a Wakanui silt loam. New Zealand Journal of Agricultural Research 48: 423-439

Brown, H.E.; Moot, D.J.; Lucas, R.J.; Smith, M. (2006). Sub clover, cocksfoot and lucerne combine to improve dryland stock production. Proceedings of the New Zealand Grassland Association, 68: 109-115

Bryant, R.H.; Parsons, A.J.; Rasmussen, S.; Edwards, G.R. (2009). Pasture production and botanical composition of high sugar and control ryegrass with or without endophyte under irrigation in Canterbury. Proceedings of the New Zealand Grassland Association 71: 177-185.

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. Grazing Management and Nitrogen excretion. Grass and Forage Science, 69, 644–654.

Buckthought, L.E., Clough, T.J.; Cameron, K.C.; Di, H.J.; Shepherd, M.A. (2016). Plant N uptake in the periphery of a bovine urine patch: determining the ‘effective area’, New Zealand Journal of Agricultural Research, 59:2, 122-140.

Burke, J.L.; Waghorn, G.C.; Brookes, I.M.; Attwood, G.T.; Kolver, E.S. (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.

Burke, J.L.; Waghorn, G.C.; Brookes, I.M. (2002a): An evaluation of sulla (Hedysarum coronarium) with pasture, white clover and lucerne for lambs. Proceedings of the New Zealand Society of Animal Production 62: 152-156

Burke, J.L.; Waghorn, G.C.; Chaves, A.V. (2002b). Improving animal performance using forage-based diets. Proceedings of the New Zealand Society of Animal Production 2002, Vol 62: 267-272.

Cameron, K.C. (1992). Nitrogen in soil. Encyclopedia of Earth System Science. Vol 3: 307-317. London, UK: Academic press.

Cameron, K.C.; Di, H.J.; and Moir, J.L. (2013). Nitrogen losses from the soil/plant system: A review. Annals of Applied Biology, 162: 145-173.

Caradus JR, Woodfield DR, Stewart AV (1996) Overview and vision for white clover. Grassland research and practice series no. 6, 1–6.

Chapman, D.F.; Kenny, S.N.; Beca, D.; Johnson, I.R. (2008). Pasture and crop options for nonirrigated dairy farms in southern Australia. 1. Physical production and economic performance. Agricultural Systems 97: 108-125.

Charlton, J.F.L.; Stewart, A.V. 1999. Pasture species and cultivars used in New Zealand - a list. Proceedings of the New Zealand Grassland Association 61: 147-166

Clark, D.A.; Anderson, C.B.; Berquist, T. (1990). Growth rates of ‘Grasslands Puna’ chicory (Cichorium intybus L.) at various cutting intervals and heights and rates of nitrogen

Clark, C.E.F.; Clark, D.A.; Waugh, C.D.; Roach, C.G.; Glassey, C.B.; Woodward, S.L.; Minnee, E.M.K,; Woodfield, D.R. (2010). Systems to increase grazeable forage production in the Waikato: A progress report on the tall fescue and perennial ryegrass component of these systems. Proceedings of the New Zealand Grassland Association 72: 49-54.

Clough, T.J.; Sherlock, R.R.; Cameron, K.C.; Ledgard, S.F. (1996). Fate of urine nitrogen on mineral and peat soils in New Zealand. Plant and Soil 178, 141-152.

Clough, T.J.; Ledgard, S.F.; Sprosen, M.S.; Kear, M.J. (1998). Fate of N-15 labelled urine on four soil types. Plant and Soil 199, 195-203.

Costall D.A.; Betteridge, K. (2010): Brief communication: Methods of delivering salt to cattle. Proceedings of the New Zealand Society of Animal Production 70: 296-298.

DairyNZ. 2012. Facts and Figures for New Zealand Dairy Farmers. Pasture and Nutrients, Version 2, 66-75.

Daly, M.J.; Hunter, R.M.; Green, G.N.; Hunt, L. (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, J.M.; Young, M.J.; Fraser, T.J.; Rowarth, J.S. (1994b). Carcass, liver and kidney characteristics of lambs grazing plantain (Plantago lanceolata), chicory (Cichorium intybus), white clover or perennial ryegrass. Proceedings New Zealand Society of Animal Production 54: 197–200

Derrick, R.W.; Moseley, G.; Wilman, D. (1993). Intake by sheep, and digestibility of chickweed, dandelion, dock, ribwort and spurrey, compared with perennial ryegrass. Journal of agricultural science 120: 51– 61.

de Klein C, Ledgard SF (2001) An analysis of environmental and economic implications of nil and restricted grazing systems designed to reduce nitrate leaching from New Zealand dairy farms. I. Nitrogen losses. N Z J Agric Res 44:201–215.

de Ruiter, J.M.; Dalley, D.E.; Hughes, T.P.; Fraser, T.J.; Dewhurst, R.J. (2007). Types of supplements: their nutritive value and use. Occasional Publication - New Zealand Society of Animal Production: 97-115.

Dhiman, T.R.; Cardorniga, C.; Satter, L.D. 1993: Protein and energy supplementation of high alfalfa silage diets during early lactation. Journal of dairy science 76: 1945-1959.

Di H.J. and Cameron, K.C. (2002a) Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. Nutrient Cycle Agroecosystems 64:237–256

Di, H.J.; Cameron, K.C.; Silva, R.G.; Russell, J.M.; Barnett, J.W. (2002). A lysimeter study of the fate of 15Nlabelled nitrogen in cow urine with or without farm dairy effluent in a grazed dairy pasture soil under flood irrigation. New Zealand Journal of Agricultural Research 45, 235-244.

During, C.; Mcnaught, K.J. (1961). Effects of cow urine on growth of pasture and uptake of nutrients. NZ Journal of Agricultural Research. 5: 591-605.

Easton, H.S.; Christenson, M.J.; Eerens, J.P.J.; Fletcher L.R.; Hume, D.E.; Keogh, R.G.; Lane, G.A.; Latch, G.C.M.; Pennell, C.G.L.; Popay, A.J.; Rolsten, M.P.; Sutherland, B.L.; Tapper, B.A. (2001). Ryegrass endophyte: A New Zealand Grassland success story. Proceedings of the New Zealand Grassland Association 63: 37-46.

Eckard, R. J.; Grainger, C.; & de Klein, C. A. M. (2010). Options for the abatement of methane and nitrous oxide from ruminant production: A review. Livestock Science, 130, 47-56.

Edwards, G.R.; Hay, M.J.M.; Brock, J.L. (2005). Seedling recruitment dynamics of forage and weed species under continuous and rotational sheep grazing in a temperate New Zealand pasture. Grass and Forage Science 60: 186-199.

Edwards, G.R.; Parsons, A.J.; Rasmussen, S.; Bryant, R.H. (2007). High sugar ryegrasses for livestock systems in New Zealand. Proceedings of the New Zealand Grassland Association 69: 161-171.

Frandson, R. D.; Wilke, W. L.; & Fails, A. D. (2006). Anatomy and physiology of farm animals. Ames, IO: Blackwell Publishing.

Fraser, P.M.; Cameron, K.C.; Sherlock, R.R. (1994). Lysimeter study of the fate of nitrogen in animal urine returns to irrigated pasture. European Journal of Soil Science 45, 439-447.

Fraser, T.J.; Rowarth, J.S. (1996). Legumes, herbs or grass for animal performance? Proceedings of the New Zealand Grassland Association 58:49-52.

Fulkerson, W. J.; Neal, J. S.; Clark, C. F.; Horadagoda, A.; Nandra, K. S.; Barchia, I. (2007). Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: Grasses and legumes. Livestock Science. 107 (2-3): 253-264

Goh, K.M., Haynes, R.J., 1986. Nitrogen and Agronomic Practice. In: Haynes, R.J. (Ed.), Mineral Nitrogen in the Plant-Soil System. Academic Press, Orlando, Florida, pp. 379-442

Hare, M.D.; Rolston, M.P.; Crush, J.R.; Fraser, T.J. (1987). Puna chicory – a perennial herb for New Zealand pastures. Proceedings of the 17th Agronomy Society of New Zealand Conference: 45-49

Harrington, K.C.; Thatcher, A.; Kemp, P.D. (2006). Mineral composition and Nutritive value of some common pasture weeds. Arable and Pastoral Weeds. New Zealand Plant Protection Society, 59:261-265.

Harris, S.L.; Auldist, M.J.; Clark, D.A.; Jansen, E.B.L. (1998a). Effects of white clover content in the diet on herbage intake, milk production and milk composition of New Zealand dairy cows housed indoors. Journal of Dairy Research 65: 389-400

Hayes, R.C.; Dear, B.S.; Li, G.B; Virgona, J.M.; Conyers, M.K.; Hackney, B.F.; Tidd, J. (2010a) Perennial pastures for recharge control in temperate drought prone environments. Part 1: Productivity, persistence and herbage quality of key species. NZ Journal of Agricultural Research, 53 (4): 283-302.

Haynes, R. and Williams, P. (1993). Nutrient cycling and soil fertility in the grazed pasture ecosystem. Adv Agron (USA) 49: 119–199.

Hoglund, J.H., Brock, J.L. (1987). Nitrogen fixation in managed grasslands. pp. 187196. In: Managed Grasslands, B. Analytical Studies. Ed. Snaydon, R.W. Elsevier Science Publications B.V., Amsterdam.

Holter, J.B. and Urban, W.E. (1992). Water partitioning and intake prediction in dry and lactating Holstein cows. Journal of Dairy Science 75, 1472–1479.

Hopkins, C. (2003). The nutritive value of Italian ryegrass (Lolium multiflorum) selected for high dry matter and nonstructural carbohydrate contents. B. Sci. Hons. University of Natal, South Africa.

Hume, D.E.; Lyons, T.B.; Hay, R.J.M. (1995). Evaluation of 'Grasslands Puna' Chicory (*Chichorium intybus* L.) in various grass mixtures under sheep grazing. NZ Journal of Agricultural Research. 38:317-328.

Hutton, P.G.: Kenyon, P.R.; Bedi, M.K.; Kemp, P.D.; Stafford, K.J.; West, D.M.; Morris, S.T. (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: 1-7.

Ivins, J.D. (1952). The relative palatability of herbage plants. Journal of the British Grassland Society 7: 43–54.

Jackson, F. S.; McNabb, W. C.; Barry, T. N.; Foo, Y. L.; Peters, J. S. (1996). The condensed tannin content of a range of subtropical and temperate forages and the reactivity of condensed tannin with ribulose-1,5-bis-phosphate carboxylase (Rubisco) protein. Proc. New Zealand Grassland Association, 57: 203-206

Kato, Y. (1946). Mechanism of uric acid excretion stimulation of aucubin. Folia pharmacol. Japon 42: 27–40.

Khelil-Arfa, H., Boudon, A., Maxin, G., & Faverdin, P. (2012). Prediction of water intake and excretion flows in Holstein dairy cows under thermoneutral conditions. Animal, 6(10), 1662-1676

Kobiyashi, H.; Takahashi, Y.; Matsumoto, T.; Nishiguchi Y. (2008). Changes in Nutritive Value of Italian Ryegrass (Lolium multiflorum Lam.) during Overwintering Period. Plant Prod. Sci. 11(2): 228-231.

Lee, J.M.; Hemmingson, N.R.; Minnee, E.M.K; Clark, C.E.F. (2015). Management strategies for chicory (Cichorium intybus) and plantain (Plantago lanceolata): impact on dry matter yield, nutritive characteristics and plant density. Crop & Pasture Science, 66: 168–183.

Ledgard, S.F.; Steele, K.W.; Saunders, W.H.M. (1982). Effects of cow urine and its major constituents on pasture properties. New Zealand Journal of Agricultural Research 25, 61-68.

Li, G.D.; Kemp, P.D.; Hodgson, J. (1997c). Biomass allocation, regrowth, and root carbohydrate reserves of Chicory (*Cichorium intybus*) in response to defoliation in glasshouse conditions. Journal of Agricultural Science. 129:447-458.

Li, G.D.; Kemp, P.D.; Hodgson J. (1997a). Herbage production and persistence of Puna chicory (Cichorium intybus L.) under grazing management over 4 years. New Zealand Journal of Agricultural Research 40, 51 –56

Li, G.; Kemp, P.D. (2005). Forage chicory (Cichorium intybus L.): A review of its agronomy and animal production. Advances in Agronomy 88, 187-222

Li, F.Y.; Betteridge, K.; Cichota, R.; Hoogendoorn, C. J.; Jolly, B.H. (2012). Effects of nitrogen load variation in animal urination events on nitrogen leaching from grazing systems. Agricultural Ecosystems and Environment 159: 81-89.

Lippke, H. and Ellis, WC. (1997). Forage quality of annual ryegrass. In: Ecology, Production, and Management of Lolium for forage in the USA. CSSA Spec. Publ. No. 24.

Mackinnon, D.; Oliver, M.; Ashton, D. 2010. Australian dairy industry: technology and management practices, 2008–09. ABARE-BRS: Canberra

Malcolm, B.J.; Cameron, K.C.; Di, H.J.; Edwards, G.R.; Moir, J.L. 2014. The effect of four different pasture species compositions on nitrate leaching losses under high N loading. Soil Use and Management 30: 58-68

McBride, S.D. (1994). Pasture yield responses to irrigation in Canterbury. Proceedings of the New Zealand Grassland Association 56: 165-168

McGowan, A.W.; Sheath, G.W.; Webby, R.W. (2003). Lucerne for high quality summer feed in North Island hill country. Legumes for dryland pastures. Grassland Research and Practice Series 11: 169-174

Menneer, J.C.; Ledgard, S.F.; McLay, C.D.A.; Silvester, W.B. (2005). The effects of treading by dairy cows during wet soil conditions on white clover productivity, growth and morphology in a white clover-perennial ryegrass pasture. Grass and Forage Science 60(1): 46-58

Mills, A.; Moot, D.J. (2010). Annual dry matter, metabolizable energy and nitrogen yield of six dryland pastures six and seven years after establishment. Proceedings of the New Zealand Grassland Association 72: 177-184.

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.

Moir, J.L.; Cameron, K.C.; Di, H.J.; Fertsak, U. (2011). The spatial coverage of dairy cattle urine patches in an intensively grazed pasture system. Journal of Agricultural Science, 149: 473-485.

Moir, J.L.; Malcolm, B.J.; Cameron, K.C.; Di, H.J. (2012). The effect of dicyandiamide on pasture nitrate concentration, yield, and N offtake under high N loading in winter and spring. Grass and Forage Science, 67: 391-402.

Moir, J. L.; Edwards, G. R.; and Berry, L.N. (2013). Nitrogen uptake and leaching loss of thirteen temperate grass species under high N loading. Grass and Forage Science 68(2): 313-325.

Monaghan, R.M.; Semadeni-Davies, A.; Muirhead, R.W.; Elliott, S.; Shankar, U. (April 2010). Land use and land management risks to water quality in Southland. Report prepared for Environment Southland.

Moorby. J.M.; Evans, R.T.; Scollan, N.D.; MacRae, J.C.; Theodorou, M.K. (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, A.J.E. and Piggot, J. (2009). The performance of pasture mixes containing 'Ceres Tonic' plantain (Plantago lanceolata) in Northland. Proceedings of the New Zealand Grassland Association, 71: 195-199.

Moot, D.J.; Brown, H.E; Pollock, K.; Mills, A. (Nov 2003). Crop growth and development affect seasonal priorities for Lucerne management. *In:* D.J. Moot (ed). Legumes for dryland pastures, Proceedings of the New Zealand Grassland Association, Symposium at Lincoln University, 201-208.

Moot, D.J.; Matthew, C.; Kemp, P.D.; Scott, W.R. (2007). Husbandry and role of pastures and forage crops in grazing systems. Occasional Publication - New Zealand Society of Animal Production: 23-33.

Nobilly, F.; Bryant, R.H.; McKenzie, B.A.; Edwards, G.R. (2013). Productivity of rotationally gazed simple and diverse pasture mixtures under irrigation in Canterbury. Proceedings of the New Zealand Grassland Association 75: 165–172.

NZIER, New Zealand Dairy Statistics, Ministry of Primary Industries Statistics New Zealand, DairyNZ Economics Group. November 2015

NZPBRA, New Zealand Plant Breeders Association. Forage trial results, Italian Ryegrass Graphs 2015.

O'connell, C.A.; Judson, H.G.; Barrell, G.K. (2016). Sustained diuretic effect of plantain when ingested by sheep. Proceedings of the New Zealand Society of Animal Production, Vol 76: 14-17.

Pacheco, D.; Waghorn, G.C. (2008): Dietary nitrogen definitions, digestion, excretion and consequences of excess for grazing ruminants. Proceedings of the New Zealand Grassland Association 70: 107-116.

Pacheco, D.; Lowe, K.; Hickey, M.J.; Burke, J.L.; Cosgrove, G.P. (2010). Seasonal and dietary effects on the concentration of urinary N from grazing dairy cows. Proceedings of the 4th Australasian Science Symposium, pp68-73.

Powell T.L. (2012) Evaluation of weighted disc meter for pasture yield estimation on intensively stocked dairy pasture. New Zealand Journal of experimental agriculture, Vol 2 Issue 3.

Powell, A.M.; Kemp, P.D.; Jaya, I.K.D.; Osborne, M.A. 2007. Establishment, growth and development of plantain and chicory under grazing. Proceedings of the New Zealand Grassland Association 69: 41-45.

Rattray, P.V. and Joyce, J.P. (1974). Nutritive Value of White Clover and Perennial Ryegrass. IV: Utilisation of dietary energy. NZ Journal of Agricultural Research, 17:401-406.

Ravera, B.L. (2014). Development of a urine harness to detect variation in urinary behaviour and urine patch coverage of dairy cows on winter crops. B.Agri.Sci (Hons). Lincoln University.

Rees, S.B.; Harborne, J.B. (1985). The role of sesquiterpene lactones and phenolics in the chemical defence of the chicory plant. Phytochemistry 24: 2225-2231

Romera, A. J., Levy, G., Beukes, P. C., Clark, D. A., & Glassey, C. B. (2012). A urine patch framework to simulate nitrogen leaching on New Zealand dairy farms. Nutrient Cycling in Agroecosystems, 92(3), 329-346.

Rumball, W.; Keogh, R. G.; Lane, G. E.; Miller, J. E.; Claydon, R. B. (1997). 'Grasslands Lancelot' plantain (Plantago lanceolata L.). New Zealand Journal of Agricultural Research 40: 373-377

Rumball, W.; Keogh, R.G.; Miller, J.E.; Claydon, R.B. (2003a). 'Choice' forage chicory (Cichorium intybus L.). New Zealand Journal of Agricultural Research 46: 49 –51.

Rumball, W.; Skipp, R.A.; Keogh, R.G.; Claydon, R.B. (2003b). 'Puna II' forage chicory (Cichorium intybus L.). New Zealand Journal of Agricultural Research 46: 53 –55

Ryan-Salter, T.P. (2011). Yield and composition of short-term pasture mixtures containing Italian ryegrass, red clover and balansa clover. B. Agri. Sci. Hons. Lincoln University.

Ryan-Salter, T.P.; Black, A.D. (2012). Yield of Italian ryegrass with red clover and balansa clover. Proceedings of the New Zealand Grasslands Association. 74: 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.

Sanderson, M.A.; Soder, K.J.; Muller, L.D.; Klement, K.D.; Skinner, R.H.; Goslee, S.C. 2005. Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. Agronomy Journal 97: 1465-1471.

Savage, J.; Lewis, C. (2005). Applying science as a tool for dairy farmers. Proceedings of the New Zealand Grassland Association 67: 61-66.

Selbie, D.R.; Cameron, K.C.; Di, H.J.; Moir, J.L.; Lanigan, G.J.; Richards, K.G. (2014). The effect of urinary nitrogen loading rate and a nitrification inhibitor on nitrous oxide emissions from a temperate grassland soil. Journal of Agricultural Science, 152: 159-171.

Silva, R.; Cameron, K.; Di, H.; Hendry, T. (1999). A lysimeter study of the impact of cow urine, dairy shed effluent and nitrogen fertiliser on drainage water quality. Australian Journal of Soil Research, 37: 357-369.

Smith, D. (1973) The non-structural carbohydrates. In: Chemistry and biochemistry of herbage. G.W Butler and RW Bailey (eds). Vo1.1. Academic Press, London, pp. 105 - 155.

Stewart, A.V. (1996). Plantain (Plantago lanceolata) – a potential pasture species? Proceedings of the New Zealand Grassland Association 58: 77- 86.

Stewart, A.; Kerr, G.; Lissaman, W.; Rowarth, J. (2014). Pasture and Forage Plants for New Zealand. New Zealand Grasslands Association, Grassland Research and Practice, Series No. 8. Fourth Edition.

Terrill, T.H.; Waghorn, G.C.; Woolley, D.S.; McNabb, W.C.; Barry, T.N. (1994). Assay and digestion of 14C-labelled condensed tannins in the gastrointestinal tract of sheep. British journal of nutrition 72: 467– 477

Thompson, D.J.; Stout, D.G.; Moore, T.; Mir, Z. (1992). Yield and quality of forage from intercrops of barley and annual ryegrass. Canadian Journal of Plant Science, 72: 163 – 172.

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

Waghorn, G.C.; Douglas, G.B.; Niezen, J.H.; McNabb, W.C.; Foote, A.G. (1998). Forages with condensed tannins – their management and nutritive value for ruminants. Proceedings of the New Zealand Grassland Association 60: 89–98

Waghorn, G.C. and Clarke, D.A. (2004). Feeding value of pastures for ruminants. New Zealand Veterinary Journal 52(6), 320-331.

Waghorn, G.C.; Burke, J.L.; Kolver, E.S. (2007). Principles of feeding value. In: Rattray, P.V.; Brookes, I.M; Nicol, A.M. eds. Pasture and Supplements for Grazing Animals. New Zealand Society of Animal Production Occasional Publication 14. In press

Welton, B.; Shorten, P.; and Costall, D. (Unpublished) Canterbury Grazing Study, Urine sensor interim results.

Whitehead, D.C. (1995a). Grasses: Uptake of Nitrogen and Effects on Morphology and Physiology. Grassland Nitrogen. CAB International, Wallingford, UK, pp. 16-34.

Whitehead, D.C. (2000). Nutrient elements in grassland. Soil-plant-animal relationships. Wallingford, UK: CAB International. pp 369

Williams, P.H.; Gregg, P.E.H.; Hedley, M.J. (1990). Use of potassium bromide solutions to simulate dairy cow urine flow and retention in pasture soils. New Zeal J Agr Res. 33:489–495

Wilman, D. and Derrick, R.W. (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: 217–223.

Woodfield, D.R. and Caradus, J.R. (1996). Factors affecting white clover persistence in New Zealand pastures. Proceedings of the New Zealand Grassland Association 58: 229–235.

Woodward, S.L.; Waghorn, G.C.; Laboyrie, P.G. (2004): Condensed tannins in birdsfoot trefoil (Lotus corniculatus) reduce methane emissions from dairy cows. Proceedings of the New Zealand Society of Animal Production 64: 160- 164

Woodward, S.L.; Waghorn, G.C.; Bryant, M.A.; Benton, A. (2012). Can diverse pasture mixtures reduce nitrogen losses? pp. 463-464. In: Proceedings of the 5th Australasian Dairy Science Symposium