Development of a urine harness to detect variation in urinary behaviour and urine patch coverage of dairy cows on winter crops

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by
Brigitte Lisa Ravera

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Dairy cow urine is the greatest contributor of nitrate leaching on agricultural systems. This is because there is a large discrepancy between the N content of grazed forages, and the N requirements of the animals; N in excess of an animal's requirement is excreted, primarily in the urine. Wintering dairy cows can contribute between 11 -24% of total farm N leaching losses, despite representing only 4-9% of the farm system. This is because of high stocking densities on winter forage crops, in conjunction with the high drainage and lack of plant uptake that occurs in winter due to high rainfall and low temperatures. While there is significant knowledge about ruminant urinary N concentrations, there is very little information available about the volume, frequency and distribution of dairy cow urine, and there is no data on these variables in winter grazing systems.

A urine harness was developed to measure the variability of dairy cow urine frequency and volume. The harness was trialled at Ashley Dene using two different wintering systems for dairy cows – kale fed at an allowance of 14kg DM cow^{-1} day^{-1} plus barley straw (3 kg DM cow^{-1} day^{-1}), and fodder beet fed at an allowance of 8kg DM cow^{-1} day^{-1}, plus ryegrass baleage (6 kg DM cow^{-1} day^{-1}). The harnesses were worn for 24 hours, and the trial was repeated three times. Urine patch area was determined by measuring *in situ* wetted area immediately following a urination event; and by regression following the development of a calibration curve between urine volume and wetted area *in situ*.

Urine harness results showed there was no difference between the two wintering systems on the frequency of urination in 24 hours (10.25 ± 2.25), or the average volume of a urination event (2.39L ± 0.29). Total daily urine volume was greater for kale (29.9L cow^{-1} day^{-1}) than fodder beet (18.0L cow^{-1} day^{-1}). The average urine patch area was 1.4 times larger on the kale treatment than on the fodder beet (0.47 vs. 0.25m^2). Total leaching losses were calculated as being greater on the fodder beet treatment than the kale (77.8 vs. 53.8kg N ha^{-1} year^{-1}).

This trial developed a satisfactory urine harness, capable of measuring urination volume and frequency, which was able to remain attached to dairy cows that were grazing winter crops *in situ* for at least 24 hours. The harness was able to show that there was great variability in urinary behaviour of dairy cows. The harness can be used, in combination with urine area to inform models used in the prediction of N losses from agricultural systems.

Keywords: New Zealand, method development, *Brassica oleracea*, *Beta vulgaris*, urine depositions, spatial coverage

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Chapter 1

Introduction

Agriculture in New Zealand, specifically dairy farming is a significant contributor to the national economy. In 2013, dairy exports were worth NZ $13.59 billion, 28% of the nation's export revenue for 2013 (StatisticsNZ, 2014). However, the drive for productivity increases and input intensification of these systems has raised concerns about the environmental impacts of nitrogen (N) pollution produced from dairy farm systems, in particular nitrate leaching losses (de Klein *et al.*, 2010).

Nitrate (NO_3^-) leaching from agricultural soils has been identified as posing major potential threats to groundwater quality in New Zealand. Increases in the concentration of NO_3^- in underground drinking-water supplies is considered a serious health hazard, as well as a factor in eutrophication. NO_3^- can interfere with the transport of oxygen in the blood, potentially causing death, in New Zealand the maximum acceptable level of NO_3^- in drinking water is 11.3 mg L^{-1} (McLaren & Cameron, 1996). NO_3^- that drains into rivers, lakes and estuaries deteriorates water quality, resulting in eutrophication (when total N concentration reaches 0.4-6 mg N L^{-1}), algal bloom and fish death (Di & Cameron, 2002).

Most NO_3^- leaching from dairy farms occurs due to high concentrations of N in the cow urine - ruminants excrete between 75-95% of the N they ingest (Eckard, Grainger, & de Klein, 2010). The reason for this is the difference between the N requirements of grazed forages and the animal N requirements, excessive N beyond animal requirements is excreted from the animal. The majority is deposited through the urine, the N in a urine patch can occur at an application rate between 800-1300kg N ha^{-1} (Eckard *et al.*, 2010). Studies have shown that between 8 and 20% of N applied in animal urine may be leached (Cameron, Di, & Condron, 2002).

Common wintering practice is to graze dairy cows on brassica crops *in situ*, this practice can contribute a disproportionate amount of whole-farm NO_3^- leaching losses; representing between 11 - 24% of total farm N leaching losses, despite representing only 4-9% of the farm system's area (Chrystal *et al.*, 2012). This is because of high stocking densities on winter forage crops, in conjunction with the high drainage and lack of plant uptake that occur in winter due to high rainfall and low temperatures.

Regional authorities have, or are, developing regional plans to manage water quality; aimed at reducing agricultural NO_3^- and other nutrient levels in surface- and groundwater (Williams *et al.*, 2013), the favoured approach is to regulate losses rather than capping nutrient inputs. The model that is most widely used to predict losses is OVERSEER®; as with all models, it has its shortcomings, and is only as reliable as the available data and information that it is modelled on.

While there is significant knowledge about ruminant urinary N concentrations, there is very little information available about the volume, frequency and distribution of dairy cow urine, and there is no data on these variables when the cows are on winter grazing systems. What is known, is that dairy cow urination events are highly variable, in both volume and frequency; studies have shown urination events can vary from 13 to 73 events in 24 hours, and total daily output from 5.8 to 54.7L (Betteridge, Andrewes, & Sedcole, 1986). The purpose of this research dissertation was to obtain data on the variability of dairy cow urination events on two different wintering systems.

Chapter 2

Literature Review

2.1 Introduction

Nitrate (NO_3^-) leaching poses potential threats to both human health and the environment. Most NO_3^- leaching from dairy farms occurs due to high concentrations of N in the cow urine. Common wintering practice is to graze dairy cows on brassica crops *in situ*, however, this practice can contribute a disproportionate amount of whole-farm nitrate leaching losses, due to high levels of drainage, lack of plant growth and high stocking densities over the wintering period. Regional authorities have, or are, developing regional plans to manage water quality, which will regulate nutrient losses from agricultural properties – these are specifically aimed at reducing agricultural nitrate and other nutrient levels in surface- and groundwater (Williams *et al.*, 2013).

This literature review covers NO_3^- in the nitrogen cycle and why it is of concern, factors that affect NO_3^- leaching, how leaching losses are predicted and the current legislature governing the acceptable levels of NO_3^- leaching from agricultural properties. In addition, the concerns surrounding the wintering of dairy cows in relation to nitrate leaching are expressed, and dairy cow water balance, and the current knowledge on urination variability is described.

2.2 The Nitrogen Cycle

Nitrogen (N) is an essential nutrient to both plants and animals; it is a vital component to the building blocks of life – amino acids, proteins and nucleic acids (Hatch, Goulding, & Murphy, 2002). Nitrogen is also a part of the chlorophyll molecule, responsible for photosynthesis, and an element in many enzymes and co-enzymes found in plants and animals (McLaren & Cameron, 1996). Probably the most important nutrient for plants, N is often one of the greatest limitations to plant growth (Hatch *et al.*, 2002) despite occurring in many different forms in large quantities in both the earth's crust (18 x 10^{15} t) and atmosphere (3.8 x 10^{15} t N_2 gas) (McLaren & Cameron, 1996). This is because typically plants are only able to take up N in certain forms, nitrate (NO_3^-) and ammonium (NH_4^+); atmospheric N_2 must first be fixed before it can be used by plants, and most N in soil is in unavailable organic form. For this reason the use of N fertilisers is one of the biggest causes of the increase in worldwide agricultural production since the Second World War (Di & Cameron, 2002); global N fertiliser consumption has increased from nearly zero in the 1940s, to 115.7 million tonnes in 2009 (FAO, 2014).

The nitrogen cycle – illustrated in Figure 2.1 – is the transfer of N from one form to another within the soil-plant-animal-atmosphere system. In addition to significant soil N input from fertiliser application, biological fixation by rhizobium in legume-based pastures and crops, organic waste application and animal manure all also contribute significant quantities of N to soil (Di & Cameron, 2002). However, as seen in Figure 2.1, not all of the N applied or fixed into the soil is taken up by plants; a large proportion is incorporated into soil organic matter, lost back to the atmosphere or leached into ground or surface waters in the form of NO_3^-.

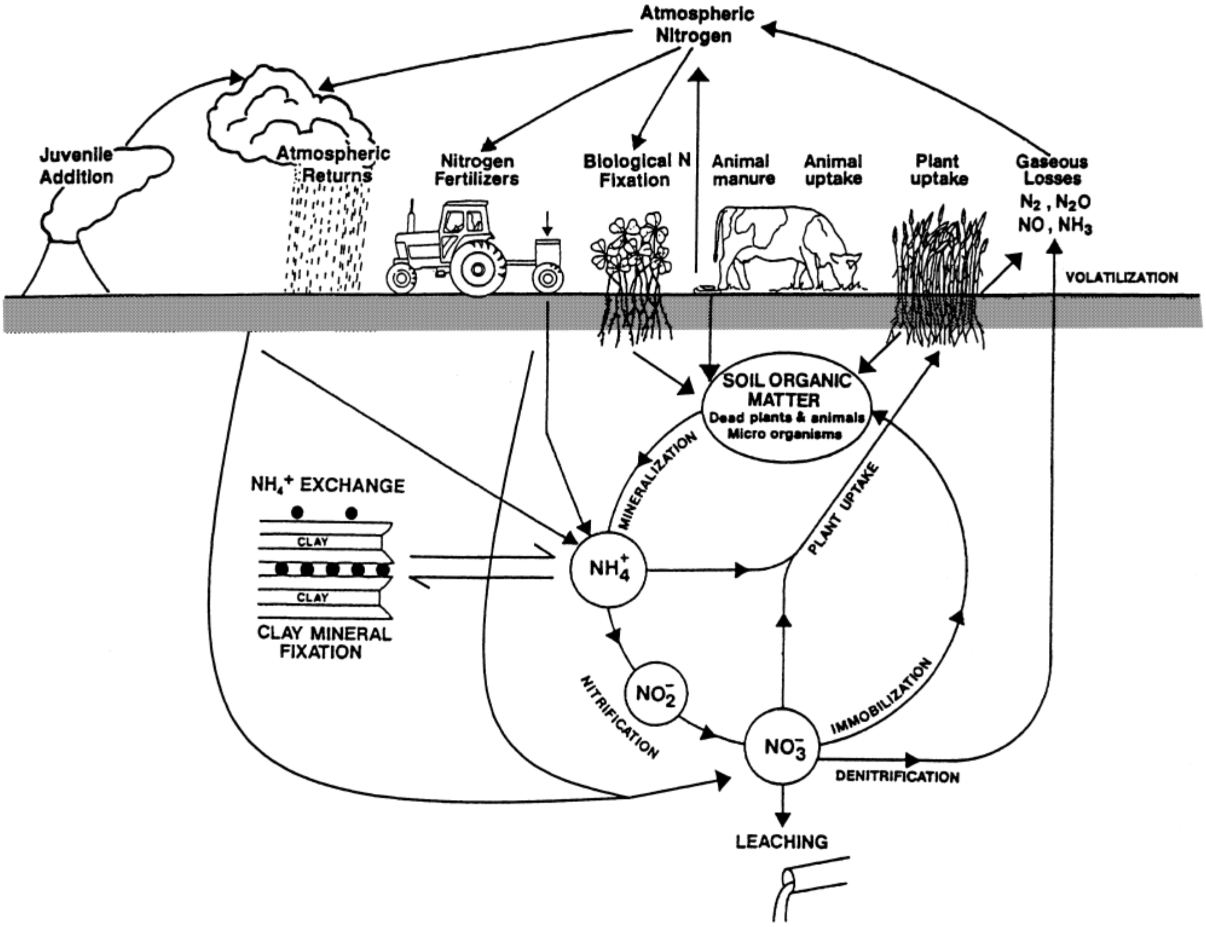

Figure 2.1 Nitrogen Cycle (Di & Cameron, 2002)

2.3 Nitrate

NO_3^- leaching from agricultural soils has been identified as posing major potential threats to groundwater quality in New Zealand (Cameron *et al.*, 2002). NO_3^- leaching can cause increases in the concentration of NO_3^- in underground drinking-water supplies. This is considered a serious health hazard, as well as a factor in eutrophication (McLaren & Cameron, 1996).

2.3.1 Nitrate impacts on health

High concentrations of NO_3^- in drinking water are considered to be harmful to human health, particularly to infants younger than 1 year of age. NO_3^- can interfere with the transport of oxygen in the blood, causing a disorder called methaemoglobinaemia (Cameron *et al.*, 2002). The ingested NO_3^- is converted in the stomach to nitrite (NO_2^-), which is rapidly absorbed into the bloodstream. The NO_2^- causes a reduction in the oxygen-carrying capacity of the blood, and potentially death from cellular anoxia (McLaren & Cameron, 1996). World health organisations have therefore set drinking water standards; the level of NO_3^--N concentration in drinking water should be no greater than 10-11.3 mg NO_3^--N L^{-1} (Di & Cameron, 2002). The maximum acceptable level in New Zealand is 11.3 mg L^{-1}(McLaren & Cameron, 1996). High NO_3^- concentrations are also toxic to livestock, causing methaemoglobinaemia and abortions in cattle, levels of 40-100 mg NO_3^--N L^{-1} are considered dangerous (Di & Cameron, 2002).

2.3.2 Nitrate impacts on the environment

NO_3^- can also cause significant environmental damage; NO_3^- that drains into rivers, lakes and estuaries can deteriorate the water quality, resulting in eutrophication, algal bloom and fish death (Di & Cameron, 2002). As the concentration of N in water increases, the water becomes nutrient rich, eutrophic; there is a gradual change in the number of plankton and other microorganisms found in water, cyanobacteria and blue-green algae populations increase and the ecosystem in the water shifts up in the trophic levels (Hatch *et al.*, 2002). Ultimately, oxygen concentrations in the water decrease leading to the death of fish. A system is considered eutrophic when the total N concentration reaches 0.4-6 mg N L^{-1} (Di & Cameron, 2002).

2.4 Factors affecting nitrate leaching

NO_3^- leaching occurs when there is an accumulation of NO_3^- in the soil that coincides or follows a period of high drainage (Di & Cameron, 2002). NO_3^- is negatively charged, as are most temperate region soils, due to same charges repelling one another, NO_3^- is not retained by soils (Di & Cameron, 2002), it is therefore readily leached when water drains through the soil. The amount of NO_3^- that is leached from the soil depends on both the NO_3^-concentration in the soil, and the amount of drainage that occurs through the soil (Cameron, Di, & Moir, 2013). The main factors affecting the level of NO_3^- leaching losses are season, climate, land use and soil properties.

2.4.1 Season and Climate

In most areas of New Zealand, the greatest NO_3^- leaching mainly occurs in late autumn, winter and early spring; this is usually when there is an excess of rainfall which is greater than the rate of evapotranspiration and occurs when the soil is already at or near field capacity (McLaren & Cameron, 1996). At these times of the year, temperature is at its annual low, and plant growth levels are minimal; plant uptake of NO_3^- is therefore also low; because of this, NO_3^- levels in the soil accumulate. In conjunction with the high rainfall and drainage experienced, NO_3^- leaching losses in winter are thus larger compared to the other seasons. For example, N leaching losses of fertiliser N applied in autumn were between 15-19%, while only 8-11% was leached from the equivalent spring-applied fertiliser (Cameron *et al.*, 2013).

Leaching can however also occur at other times of the year if the soil is close to field capacity and an event of heavy rainfall or irrigation occurs. A dry summer can also create leaching problems later on in the year; a dry summer could cause accumulations of NO_3^- in the soil due to low plant uptake (plant growth limited by water availability), which then results in higher than average leaching losses in the following winter (Cameron *et al.*, 2002). This is illustrated in Figure 2.2, in all four treatments, as the soil water deficit increases the level of NO_3^- leached increases.

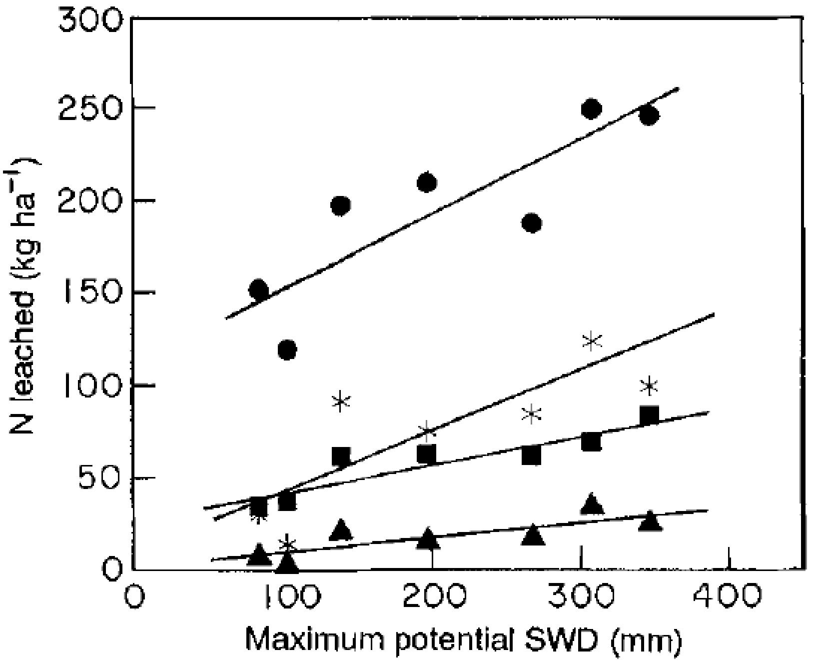

Figure 2.2 Linear regressions of nitrate leaching from the old swards on the maximum potential soil water deficit.●, 400N drained, *y*= 112.9+0.3*x*, r^2=0.73; ✳, 400N undrained, *y*= 10.3+0.31*x*, r^2=0.67; ■, 200N drained, *y*= 30.6+0.13*x*, r^2=0.64; ▲, 200N undrained, *y*= 2.2+0.07*x*, r^2=0.50 (Scholefield *et al.*, 1993)

2.4.2 Land Use

In general, leaching losses increase as the land use intensifies (McLaren & Cameron, 1996). The risk of NO_3^- leaching losses increases exponentially with the total amount of N input, regardless of what type of N input occurs (de Klein *et al.*, 2010); as systems become more intensified, inputs increase. In undisturbed, natural ecosystems like forests, leaching is usually low; extensively grazed pastures can also have low leaching losses. However, intensively grazed pastures – particularly dairying systems –

are typically high sources of NO_3^- leaching, especially when high stocking rates are combined with high fertiliser inputs. High NO_3^- leaching in these intensive pasture grazed systems is largely due to the amount of N contained in ruminant urine and dung. NO_3^- leaching in arable systems is highly variable and largely depends on the amount of fertiliser and irrigation used, and the type of crop itself (McLaren & Cameron, 1996). Table 2.1 illustrates some examples of the differences between different agricultural land uses; as is evident, dairy farming had the highest level of leaching loss (110 kg N ha^{-1} y^{-1}) when N was applied at 360kg ha^{-1}, while the lowest leaching loss (6 kg N ha^{-1} y^{-1}) occurred on the sheep farming system. This is a good example of the differences that occur in NO_3^- leaching levels based on the intensity of the system – a dairy system with annual N fertiliser inputs of 360kg ha^{-1} is a highly intensified system, while a sheep farming operation is generally an extensively grazed operation.

Table 2.1: Examples of NO3-N leaching losses under grazed pasture and arable systems (Cameron *et al.*, 2013)

Land Use System	Soil texture	N applied (kg N ha^{-1} y^{-1})	Leaching loss (kg N ha^{-1} y^{-1})
Dairy	Silt loam	0	25
Dairy	Silt loam	360	110
Cattle	Clay loam	0	30
Cattle	Clay loam	400	56
Sheep	Sandy loam	0	6-7
Sheep	Sandy loam	400	11-41
Cropping – Cereal rotation	Silty clay loam	0	8
Cropping – Cereal rotation	Silty clay loam	288	58
Mixed cropping: autumn ploughing, winter wheat	Silt loam	125	14-102

2.4.3 Soil texture

Soil texture and structure affect the rate at which water drains through a soil, and therefore affect the rate at which NO_3^- is leached from the soil (McLaren & Cameron, 1996); the more rapid the rate of leaching, the less opportunity there is for plant uptake, denitrification or immobilisation to remove the NO_3^- from the soil solution (Cameron *et al.*, 2002).

Rapidly draining soils are prone to higher levels of NO_3^- leaching losses than slower draining ones under the same conditions. Losses are generally greater in soils with poorly structured sands than in clay soils because of slower water movement in the latter soils. Sandy soils have lower field capacity than clay or silt loam soils, and therefore leaching is induced more quickly in these lighter soils(Cameron *et al.*, 2002). In addition to soil texture, the soil pore proportions can also influence the rate of leaching. Higher proportions of macropores in the soil – caused by earthworms, plant roots or wetting and drying cycles - mean that water moves more rapidly through these soils (Cameron *et al.*, 2013). Agricultural drainage systems have also been shown to increase the level of nitrate leaching that occurs in soils. Table 2.2 illustrates how paddocks with molepipe drainage consistently had significantly higher levels of annual NO_3^- losses on a continuously grazed beef system.

Table 2.2: Losses of nitrate-N by leaching (kg ha^{-1}) (Scholefield *et al.*, 1993)

	1983-84	1984-85	1985-86	1986-87	1987-88	1988-89	1989-90
Undrained	32.6	61.9	42.0	19.2	39.5	5.6	57.7
Drained	113.6	136.6	113.0	79.0	127.0	81.8	168.8

2.5 Ruminant urine excretion: Dietary N and urinary N output

The two main N inefficiencies in grazed dairy systems are the relatively high N requirements in pasture plants for optimum growth compared to the dietary N requirement of cows, and the high concentration of excreted N in urine patches (Ledgard *et al.*, 2000). Like plants, N is also an essential nutrient for animals; an insufficient supply of dietary N relative to requirements can limit ruminant production (Pacheco & Waghorn, 2008). N requirements of dairy cattle are 1.8% of DM, 2.2% for growing cattle and 3% for young or lactating animals (Pacheco & Waghorn, 2008). However, grazed dairy pastures typically have N concentrations exceeding 3.4% of DM (de Klein *et al.*, 2010).

Ruminants excrete between 75-95% of the N they ingest (Eckard *et al.*, 2010), depending on the feed source. The majority of the excreted N is deposited through the urine – a urine patch can have an application rate between 800-1300kg N ha^{-1} (Eckard *et al.*, 2010). This excessively high concentration is primarily due to the aforementioned mismatch between the N requirements of grazed forages and the animal's requirements.

Increases in N intake generally lead to considerable increases in N loss as higher concentrations of N in the urine - the critical point in relation to the primary form of N excreted is an intake of 400g N day^{-1} (Castillo *et al.*, 2000). Figure 2.3 illustrates how increases in the N intake increase the N output in urine, particularly beyond 400g N day^{-1}, which results in exponential increases. Faecal output remains at a constant gradient; Castillo *et al.* (2000) suggest this constant is around 7.5g N per kg of DM ingested.

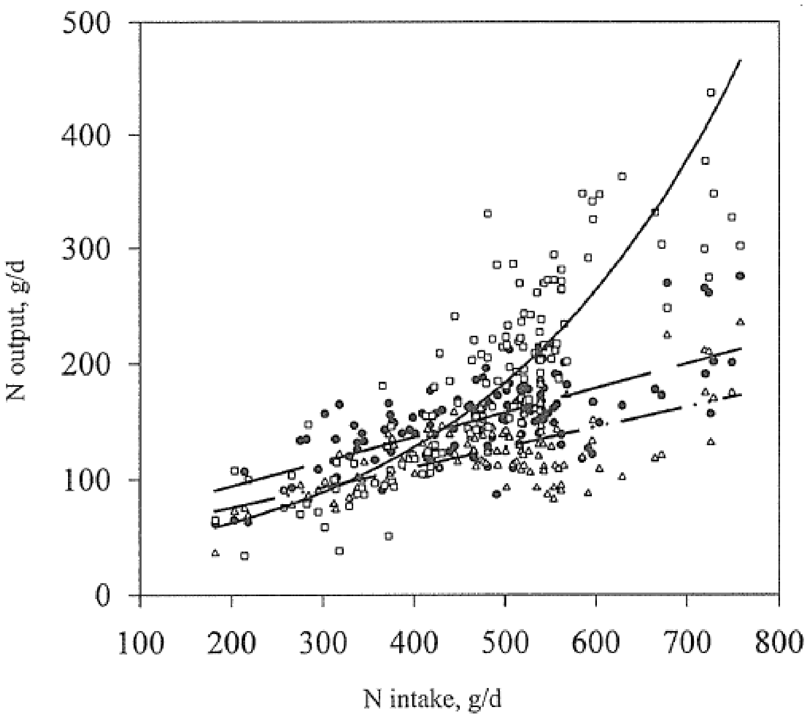

Figure 2.3 Relationship between total nitrogen intake (g d^{-1}) and output (g d^{-1}) in faeces (●), urine (☐), and milk (△). (Castillo *et al.*, 2000)

Studies have shown that between 8 and 20% of N applied in animal urine may be leached (Cameron *et al.*, 2002), which is highly significant when the N concentration in these patches is between 800-1300kg N ha^{-1}. Figure 2.4 illustrates how on a typical dairy farm the primary driver of N leaching is from grazing, i.e. animal urine, rather than the application of fertiliser or effluent. The main effect of fertiliser N use on N losses from grazed pastures is indirect – with higher fertiliser N inputs pasture production and animal stocking rate is increased, and thus urine N excretion rates are increased.

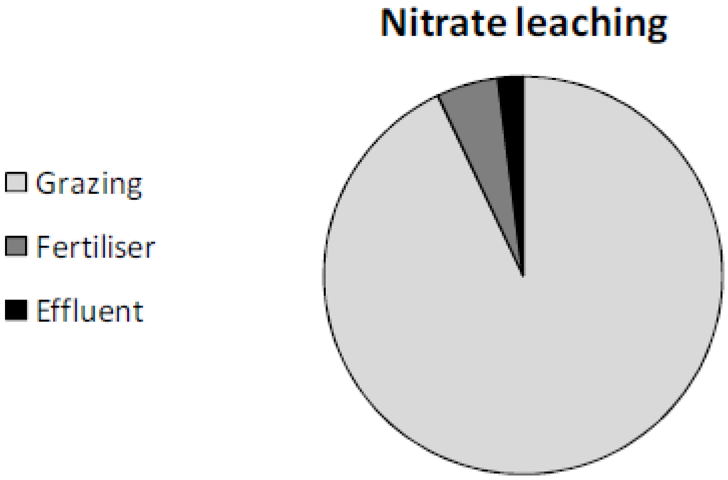

Figure 2.4 Relative contribution of N sources of N leaching losses for a typical NZ dairy farm (de Klein *et al.*, 2010)

2.6 Challenges of wintering dairy cows in relation to nitrate leaching

The main problem encountered by dairy farmers in relation to wintering their cows, particularly in the South Island, is the inability to grow sufficient pasture during winter to meet the daily feed energy requirements of the animals (Dalley, 2011); common practice is therefore to graze the dairy cows on brassica crops *in situ* (Chrystal *et al.*, 2012). This practice can contribute a disproportionate amount of whole-farm nitrate leaching losses; representing between 11 -24% of total N leaching losses, despite representing only 4-9% of the farm system area (Chrystal *et al.*, 2012).

As covered in Section 2.4, factors of temperature, rainfall and soil strongly influence the level of NO_3^- leaching that occurs; winter forage crop leaching losses are exacerbated by the high density of urine patches associated with high stocking rates on crops, low plant growth rates, and the influence of heavy soil drainage systems (Dalley, 2011). Figure 2.5 illustrates how NO_3^- leaching losses on grazed winter forage crop systems are high compared to losses measured under pasture. This trial modelled six farm systems each with a different wintering system, the diagonally checked bars represent the typical system where stock are grazed over the entire winter period on a winter forage crop. It is evident from these results that the greatest leaching losses occurred on the winter crop areas. For the typical system this was modelled at around 58kg N $ha^{-1}year^{-1}$, this was nearly 2.5 times the level leached from the main farm block (23kg N/ ha/year). In fact, in all systems where the cows grazed a winter crop, NO_3^- leaching was over double the leaching level.

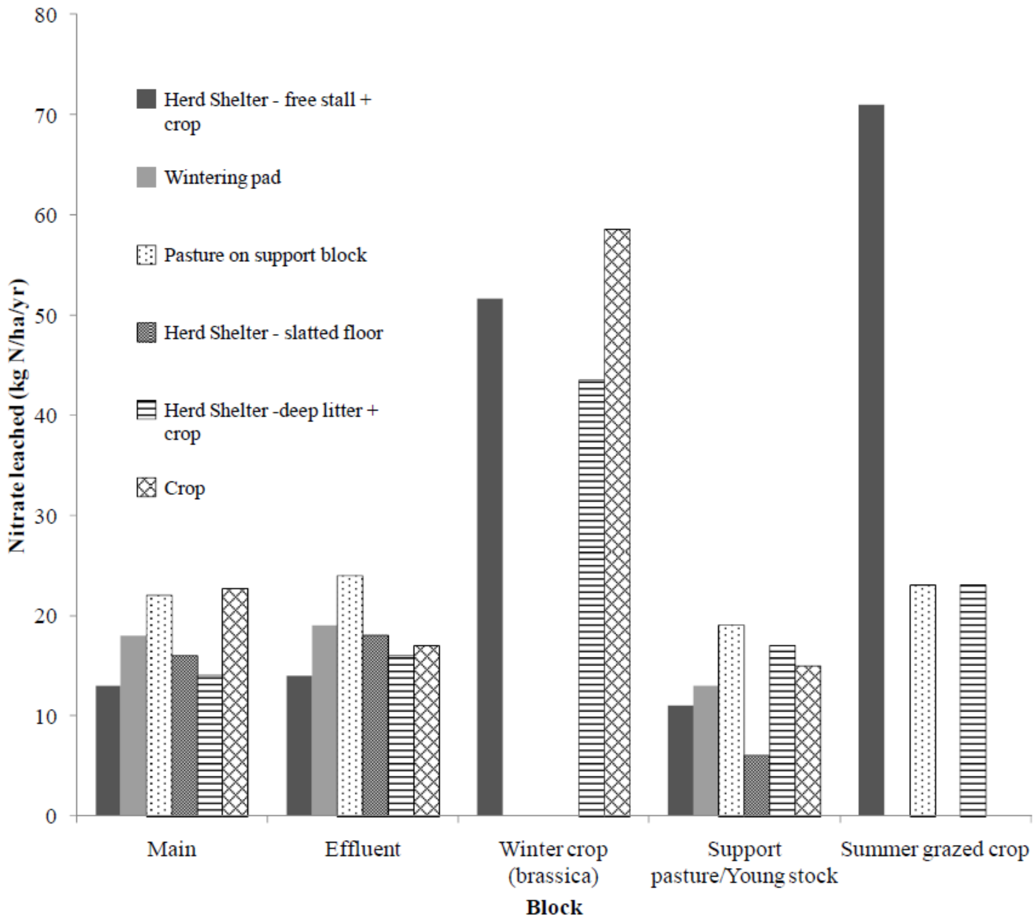

Figure 2.5 Modelled N leaching losses from each of the monitor farm blocks (kg N/ha/yr) (Chrystal *et al.*, 2012)

2.7 Predicting nitrate leaching losses

In response to the NO_3^- leaching losses from agricultural land, the National Policy Statement for Freshwater Management, which came into effect 1 July 2011, mandates that regional authorities across New Zealand must set and manage land uses within water quality limits (Williams *et al.*, 2014). These authorities have, or are developing regional plans to manage water quality; aimed at reducing agricultural NO_3^- and other nutrient levels in surface- and groundwater (Williams *et al.*, 2013).

2.7.1 Predicting leaching losses

The favoured approach with these regional plans is to regulate losses rather than capping nutrient inputs. However, determining nutrient losses is significantly more difficult than monitoring inputs (Williams *et al.*, 2013). There are essentially two approaches that can be taken to calculate nutrient budgets: measurement and modelling. Routinely measuring nutrient losses at a farm or paddock level is currently impractical – these methods are often not spatially or temporally suitable for the monitoring requirements, and they can be costly, time consuming and generally have very large variability (Cichota & Snow, 2009). The alternative to measurement is to use computer simulation models which are based on knowledge of the processes involved and available data to simulate the farming system.

There are a number of different models available for calculating nutrient balances, the main difference between them is how items within the balance are estimated. Some models use complex mechanistic or process-orientated descriptions of the large number of processes involved in nutrient dynamics (Cichota & Snow, 2009); others use simpler, empirical, descriptions of the processes and take fewer factors into account. These simpler models are usually associated with large spatial and temporal scales, such as average annual losses on a paddock, farm or in a catchment. One of these models that is widely used for estimating nutrient budgets is OVERSEER®.

Many of the regional plans that have been developed for managing nutrient losses include use of the OVERSEER® model for estimating NO_3^- losses from individual pastoral farms. At present, Environment Canterbury, Otago Regional Council, Environment Southland, Waikato Regional Council, and Environment Bay of Plenty specify the use of OVERSEER® for recording estimated nutrient losses from individual properties. Other regional authorities are also investigating using OVERSEER® (Williams *et al.*, 2013).

2.7.2 OVERSEER®

OVERSEER® was originally developed to guide nutrient management in pastoral farms - its initial purpose was to assist with fertiliser management (Cichota & Snow, 2009; Williams *et al.*, 2013). It has now evolved to become a tool for evaluating farm systems, including their impact on the environment (Cichota & Snow, 2009); as mentioned, in recent times it has been used to monitor farm nutrient losses as an instrument for environmental policies.

The OVERSEER® model uses empirical relationships, internal databases and readily available data from an 'existing' farm to estimate the nutrient inputs and outputs at farm or paddock level, presenting them in a nutrient budget (Cichota & Snow, 2009). It does not simulate production, but rather requires information on farm productivity and farm inputs (fertilisers and supplements) (Cichota & Snow, 2009). It was designed to predict the long-term average behaviour of the system and is not suitable for examination of extreme-case scenarios or systems in transition.

The model was developed reviewing the knowledge obtained primarily in New Zealand, and in consultation with its end-users (farmers and consultants), it is therefore highly suited to the management practices and environmental conditions of New Zealand. OVERSEER® was specifically designed to require minimal inputs with data that are significant and easily obtained by farmers (Cichota & Snow, 2009; Williams *et al.*, 2013).

It is important to note that models are only simplified descriptions of natural systems, and that all of them have limitations. Their performance is often restricted because we have incomplete knowledge of how natural systems and processes operate and assumptions need to be made by the model. For this reason, there will always be uncertainty in all modelling efforts (Cichota & Snow, 2009). While a model's ability to make predictions may be limited, as models are updated with better understanding of the processes and systems involved, reliability will improve.

2.8 Current knowledge on urination variability

While many N cycling models use average values for describing urine excretions as their input data, the actual concentration and volume of individual urination events can vary greatly. There is currently limited data available on the volume, frequency and distribution of dairy cow urine, and no data exists for these variables in wintering systems. Limited research has been conducted on urinary output where the cows are actually grazing in the field as opposed using to metabolism crates.

What is known, is that urination events vary greatly. Betteridge, Andrewes & Sedcole (1986), in a study on grazing steers found that with a 24 hour period, frequency of urination varied from 13 to 73 times, and total daily output ranged between 5.8 and 54.7 L. Urinary N concentration ranged between 0.8 to 14.1 mg N/L. In a more recent study (Betteridge *et al.*, 2013) with pasture grazed dairy cows, the average volume of a dairy cow urination event was found to be 2.1L, but volumes ranged between 0.30 to 7.83 L event^{-1}. Average urinary N was 9.5g N L^{-1}, ranging from 1.2 to 24.7g N L^{-1}. Other studies found frequency of urination by cows were 7 and 9.3 per 24 hours (between 2-18 and 3-19) (Villettaz Robichaud *et al.*, 2011), and 8.95 urinations per 24 hours (Aland, Lidfors, & Ekesbo, 2002), however in both of these studies the cows were housed.

2.9 Water balance in cows

Water constitutes about 60% of an animal's mass, despite contributing no energy it is the most essential component of the diet (Keenan, 1988). Dairy cows require water for all their life processes - to maintain osmotic pressure in cells and tissues, to eliminate waste materials, and to dissipate excess heat from the body (Khelil-Arfa *et al.*, 2012). Water is constantly being lost from the body, and it must be replenished if the animal is to remain in water balance and not become dehydrated. Most is lost via the urine, but it is also lost in the faeces and by evaporation from body surfaces, such as the skin and respiratory passages (Frandson, Wilke, & Fails, 2006). Water in the body must be maintained through water intake from drinking, water contained in feed consumed and water produced from metabolic pathways (Khelil-Arfa *et al.*, 2012).

Urine formation occurs in the kidneys; the kidneys regulate the body balance of not only water, but also various electrolytes, acids and bases by adjusting the volume and composition of urine in response to changes in dietary intake or metabolism (Frandson *et al.*, 2006).

The volume of urine produced is primarily determined by the mineral load that needs to be excreted. Animals fed a high protein diet consume more water and excrete more water in urine (Bannink, Valk, & van Vuuren, 1999). This theory is confirmed in other studies, Khelil-Arfa *et al.* (2012), found that the content of crude protein (CP) ingested in daily feed intake was the principal factor affecting the volume of urine, an equation produced based on CP produced an R of 0.77. Holter and Urban (1992) also found that urine output was affected by dry matter intake (DM), dietary DM%, and dietary CP (R^2=0.92). In this study as well, dietary CP was the factor that had the greatest effect. In addition to N, urine production is also particularly affected by the excretion of Na and K (Bannink *et al.*, 1999).

2.10 Conclusions

NO_3^- leaching is of significant concern to dairy farming systems in New Zealand. High levels occur due to high concentrations of N in the cow urine. Wintering practices of grazing cows on brassica crops *in situ* can contribute a disproportionate amount of whole-farm NO_3^- leaching losses; representing between 11 -24% of total farm N leaching losses (Chrystal *et al.*, 2012). Regional authorities have, or are, developing regional plans to manage water quality - aimed at reducing agricultural nitrate and other nutrient levels in surface- and groundwater. These plans regulate nutrient losses; in order to estimate the levels of losses, computer simulation models are used, the most widely used model is OVERSEER®. All models have shortcomings, and are only as reliable as the available data and information that they are modelled on. Dairy cow urination events are highly variable, both in volume and frequency, for example one study found cows urinated between 13 to 73 times in 24 hours and the urination volumes ranged from 0.3 to 7.83L per event (Betteridge *et al.*, 2013). Urine formation occurs in the kidneys which regulate the body's water balance by adjusting the volume and composition of the urine; the CP content of dietary intake is the key factor affecting this. The purpose of this research dissertation is to provide quantitative data on the variability of dairy cow urination events on two different wintering systems, hopefully so that greater accuracy in the models used to predict nutrient losses can be achieved.

2.11 Research Objectives

The objectives of this research trial were to:

- Develop a harness system capable of capturing data on cow urinations: volume and frequency

- Compare two wintering systems using the urine harnesses to determine whether dairy cow urine volume, frequency and nitrogen content change based on the type of system used

- Compare the two wintering systems to determine whether there is a difference in the area of the urine patches produced.

- Use the collected data to estimate NO_3^- leaching losses on the two wintering systems

This will allow for better modelling to be developed on the levels of NO_3^- leaching, as well as allowing more detailed strategies for minimising leaching to be developed.

Chapter 3

Materials and Methods

3.1 Method Development

3.1.1 Development of Urine Harness

The design of the urine 'harness' is relatively simple; essentially consisting of a thick reinforced rubber glove (neoprene outer layer) which was superglued (Henkel's Loctite Power Flex Gel) to fit into a pointed oval shaped hole in a piece of vinyl covered upholstery fabric (16.5cm x 11.5cm). All but one finger of the rubber glove were excluded by knotting tightly with string. The remaining finger was attached to a pipe connector piece using a cable tie – this pipe connector was used to attach to the flow meter sensor. Plate 3.1 illustrates some of the earlier prototypes trialled, where different glove materials and sizes of fabric area were experimented with. Part C shows the final prototype which was very satisfactory. The final design was found to improve the accuracy of the flow sensor, as it channelled the urine through it with less vortexing. Vortexing draws air through the sensor, and air mixed with water changed the calibration of the sensor


A B C

Plate 3.1 Earlier prototypes of the final harness design (A & B) and the final prototype (C)

The 'harness' was attached to the rear of the cow using superglue (Henkel's Loctite Power Flex Gel) on the fabric part of the harness, the vulva fits into the gloved funnel. The non-toxic superglue was approved for use on animals. Ordinary strapping tape was also used around the edges of the fabric to provide extra adhesion to the cow and protect the edges of the fabric from starting to pull away from the hide (Plate 3.1 B and C). Once the cow was fitted into the harness, the flow meter sensor was screwed into the open finger pipe connector. The flow meter sensor design was U-bend shaped to maintain a constant ‘slug’ of liquid through the sensor turbine and to prevent air pushing through it - air that mixed with liquid in the sensor created huge variation in the results (up to 100% variation), liquid alone was less chaotic and caused no turbulence through the turbine.

Calibration of the device was done in the laboratory by tipping known quantities of water into the urine collection harness with sensors attached, at different speeds. The logger counted the pulses from the sensor and also timed the period over which the sensor was active. The average number of pulses for 1L was 58.2. From this, the sensor pulses were regressed against water volume to create a linear calibration used in the final logger programme. The calibration was only accurate within a certain flow rate range; the range was selected based on flow rates expected from cows. The flow rate range was less than or equal to 10 seconds to pass around 1L through the glove. If the flow rate falls within this range, the CV is ~10%; outside of this range, error of measurement increases.

In addition to the harness, the cows were also fitted with custom-made covers (see Plate 3.2). These covers had pockets either side of the shoulders and a Velcro strip running along the length of the animal’s back. The pockets were for the radio logger device and a counter weight to be carried by the animal; the Velcro strap was for the wire that runs from the flow meter sensor to the radio logger. The cows were acclimatised to the covers by randomly selecting a number of cows to be fitted into them one week prior to the trial start date; this enabled both the animals wearing covers, and other cows in the paddock, to become accustomed to the covers.

Plate 3.2 Cows wearing the entire harness device

3.2 Validation of Harness and Calibrations

3.2.1 Experimental site

The trial had two parts to it; firstly, actual real-time urine frequency and volume data was collected, and then, urine patch area data was collected. The validation experiment, which involved using animals and diets from a larger study (Pastoral 21: Phase II), was conducted with the approval of the Lincoln University Animal Ethics committee. Both parts of the experiment were conducted at Lincoln University's Pastoral Research Farm, Ashley Dene's, main block; located at -43.65° North, 172.33° East, on Lismore/ Balmoral shallow stony loam soil structure. There were two forage crop systems which included one 3ha paddock of late kale (cultivar "Regal), sown at a rate of 4kg ha^{-1}, and 1ha paddock of fodder beet (cultivar "Rivage") sown at a rate of 104, 000 plants ha^{-1}. Plate 3.3 is an illustration of Ashley Dene indicating on which paddocks the trial took place. Fodder beet was sown after conventional ploughing and cultivation procedure, and the kale was sown into oat (*Avena sativa* L.) stubble by direct drill. The crops were maintained under lateral spray irrigation during the growing season. The kale received 200 and 15kg/ha of diammonium phosphate and boron (10% B) at sowing, and with 102kg N/ha urea. Fodder beet received 250, 350, 200 and 15kg/ ha respectively CropMaster 20, sodium chloride, potassium chloride and boron (10% B) at sowing, it also received 170kg N/ha as urea in two split treatments throughout the growing season. The fodder beet also was placed on a spray rotation, receiving two applications (Nortron + Bentanal Forte + Goltix).

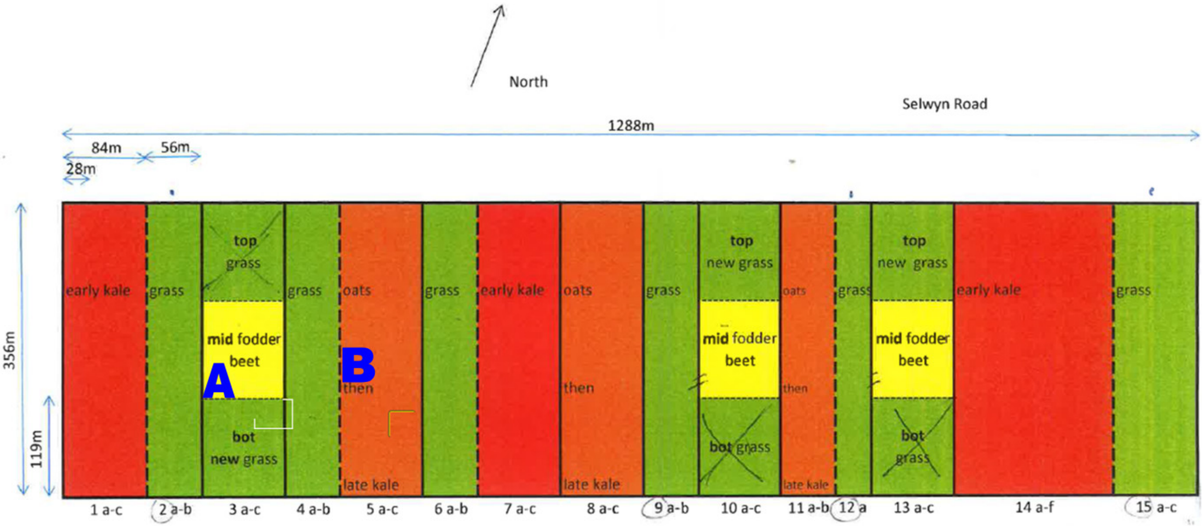

Plate 3.3 Site map of Ashley Dene. The A indicates the site of the fodder beet treatment. The B indicates the site of the kale treatments.

3.2.2 Experimental Design

The experimental design was a two factor, replicated completely randomised design. The design was made up of two treatments -two dairy wintering systems, (i) wintered on kale and straw and (ii) wintered on fodder beet and grass baleage.

3.2.3 Animals/ Management

All animals used in the trial were from the Lincoln University Research Dairy Farm. There were 22 dairy cows for the entire trial, split equally into one of the two treatment groups, and wintered on either fodder beet or kale. The kale cows were fed an allowance of 14kg DM cow^{-1} day^{-1}, plus barley straw (3 kg DM cow^{-1} day^{-1}) supplying approximately 180 MJ ME cow^{-1} day^{-1}, and 350 g N cow^{-1} day^{-1}. The fodder beet cows were fed an allowance of 8kg DM cow^{-1} day^{-1}, plus ryegrass baleage (6 kg DM cow^{-1} day^{-1}) supplying approximately 186 MJ ME cow^{-1} day^{-1}, and 240 g N cow^{-1} day^{-1}. Stocking rate was equivalent to 50 cows per 1 ha on the fodder beet and 50 cows per 3ha on the kale.

The cows were selected based firstly on whether they were fistulated, and then on age. The average age of the animals used in the trial was 6.4 years old. The rationale behind the decision to choose fistulated and older research farm cows was because these were the animals which would adjust more readily to being handled. The average liveweight of the animals selected for the trial was 502kg, and average BW was 97. Animals sampled in each of the urine harness trials were randomly selected from the available trial cows in each treatment. For the urine patch area observations, urine patches from any cows in the two treatment paddocks were used.

3.2.4 Urine N Concentration

Urine sampling was performed in both June and July on two mobs of cows from the research dairy farm. These mobs received the exact same treatments as described above (feeds and allowances); there were 34 cows sampled on the fodder beet treatment, and 28 cows sampled on the kale treatment. Urine samples were taken mid-stream after manual stimulation of the vulva, then acidified below a pH of 4.0 using concentrated sulphuric acid to prevent volatilization, and then frozen until analysis at -20°C. Urine N% was determined using an N-analyser (Vario MAX CN, Elementar Analysensysteme, Hanau, Germany).

3.3 Part 1: Urine Harness Trial

This part of the research trial was run from the 9th to the 17th of July 2014. The trial was repeated on three occasions, the first trial ran for 48 hours from 9th July to 11th July. The next two repetitions ran for 24 hours each, on 14-15th July, and 16-17th July. In the first two trials, three cows from each treatment were randomly selected and attached to the harness system. In the final trial, 5 random cows from each treatment were used. The urine harnesses recorded urination volume and frequencies, automatically radio logging the data.

After the trial, the results were filtered to only provide what were considered 'true urination events'. Most data filtering was simply taking out values of <4mL minute^{-1}; these apparent, very small amounts could have been the result of a signal being transmitted simply due to the shaking of the sensors as the animals walked. Results that were excessively large volume amounts were also removed; these were caused when the cows escaped from the cover or some other unusual event occurred. Events such as these caused recordings of very large amounts, e.g. 80L urinations, which were deemed to be unrealistic. Where it was known the device failed – either by failing to remain stuck to the animal for a full 24 hours, or there was an issue with the actual sensor device itself, the results were also removed. Additionally, where the data showed more than one recording within a minute, or immediately after, these recordings were combined. If the recordings were identical, one was deleted as this was due to a glitch in the programming of the data loggers.

Plate 3.4 The urine harness in action as a cow urinates through it

3.4 Part 2: Urine Patch Area Observations

3.4.1 Urine Patch Wetted Area Data Collection

These measurements were carried out between the 16th-20th June 2014. In total, 169 urine patch area measurements were taken; 83 were taken from the fodder beet treatment, and 86 from the kale treatment cows.

The method of obtaining this data involved observing cows urinating in treatment paddocks where they were feeding. Once a cow urinated, observers would walk over to the urine patch and outline the wetted area using spray paint. A 1m ruler was placed on the ground on top of the patch, then using a digital camera, held parallel to the ground, a photo was taken of the patch (Plate 3.5). Care was taken to ensure all patch edges and the entire ruler were included in the shot.

Plate 3.5 Examples of urine patch wetted area photos

3.4.2 Calibration Curve

Following the collection of the observed urine patch area photos, a series of calibration photos were taken of the soils in each of the treatment paddocks. The purpose of this was to try to create a calibration curve describing the relationship between urine volume and urine patch wetted area so that volumes could be calculated for the collected areas. This was achieved by applying a range of simulated urine volumes to the soil and then measuring the surface wetted area using photographs and computer analysis. The calibration curve data was collected on the 23 and 24th of June 2014.

These measurements were taken on the fodder beet and kale paddocks separately. A known volume of trough water was poured – at the height of a cow's vulva – onto the soil. The wetted area was then outlined and photographed using the same protocol described in Section 3.4.1. There were 10 simulated volumes used: (i) 0.5L, (ii) 1.0L (iii) 1.5L, (iv) 2.0L, (v) 2.5L (vi) 3.0L, (vii) 3.5L, (viii) 4.0L, (ix) 4.5L, and (x) 5.0L. Each volume was replicated 5 times.

3.4.3 Computer Analysis to Calculate Area

All urine patch observation photos were analysed using an irregular area calculator – SketchAndCalc^{TM} – which is online-based software. The scale of the photo was set using the ruler in the photo, as the length of this was known to be 101cm. The patch outline was then freehand drawn on the inside of the spray paint outline. SketchAndCalc^{TM} then calculated the area of the patch in cm^2.

3.5 Leaching loss calculations

Paddock leaching losses were calculated using the following equation:

$$\mathbf{N_L = (N_{L1} \times P_1) + (N_{L2} \times P_2)}$$

N_L = annual average NO_3-N leaching losses from a grazed field

N_{L1} = N leaching losses at the urine patch

N_{L2} = N leaching losses at non-urine patch areas

P_1 = proportion of area covered by urine patch areas

P_2 = proportion of area covered by non-urine patch areas

Estimates of leaching losses were calculated using lysimeter data collected at the site in 2013 (K. Cameron, unpublished data). N leaching losses used for non-urine patch areas were 9.9 kg N ha^{-1} for the kale treatment, and 11.5kg N ha^{-1} for the fodder beet treatment, measured in 2013 (K. Cameron, unpublished data).

N leaching losses for urine patch areas were calculated by first calculating the N load per average urination, and then multiplying this by the percentage of N load leached on that treatment. The equation used to determine urine patch N load was:

Urine patch N load = $\frac{\text{(Urine N concentration x Average urination volume)}}{\text{Calculated verage urine patch area}}$

The percentage of the N load that would be leached was taken from 2013 lysimeter data at the site; these values were was 32.16% on the fodder beet treatment, and 33.53% on the kale treatment (K. Cameron, unpublished data).

The proportion of area covered by urine patches was calculated using the following equation:

Urine patch coverage (%) = (average number of urinations/24hrs x average area per urination x number of cows x number of days on paddock) ÷ total area grazed

The proportion of non-urine patch area was calculated by subtracting the value calculated above from 1. These values were then substituted into the initial equation described to calculate total paddock leaching losses.

3.6 Statistical Analysis

Results were analysed using Excel and GenStat 16.

For the urine harness data, Excel was used to calculate the means of urination frequency, urination volume, total daily volume, and volumes per kg liveweight. Where a cow was used in more than one trial, that cow's results were averaged, as each individual cow represented a separate replicate. GenStat was used to run one-way ANOVAs to test whether the results between treatments were statistically different.

Excel was also used to calculate a regression equation for the urine patch calibration treatments. The equations were then used to calculate the volumes of all observed urine patch areas. Excel was used to calculate the means, variance, standard deviation and standard errors. GenStat was used to run a T test to analyse the two means.

Chapter 4

Results

4.1 Climate

Daily rainfall and air temperatures for the study period are shown in Figure 4.1. Precipitation in both June and July (43.4mm and 48.3mm) was below the 31 year monthly total precipitation averages, (57.2mm and 57.8mm respectively) (NIWA, 2007). Mean air temperature during June and July was 8.2 and 7.0°C; this higher than the 31 year average temperatures of 6.7 and 6.1°C for these months.

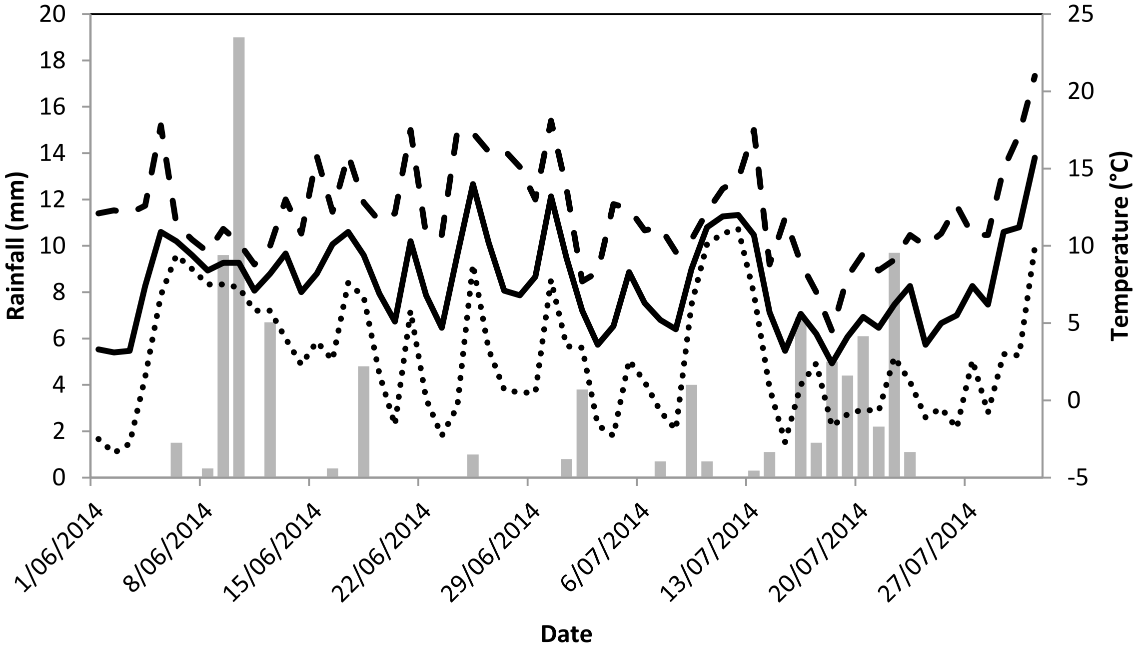

Figure 4.1 Total daily rainfall (solid gray) (mm) and daily air temperatures: maximum (dash), mean (solid), and minimum (dot) (°C) for June and July 2014. Figure is calculated from Lincoln Broad Fields weather station (-43.63°N and 172.47°E), 11.2km from Ashley Dene main block (NIWA, 2007)

4.2 Urine N Concentration

Urine N concentration (g N L^{-1}) is shown in Table 4.1. The cows on the kale treatment had 1.2 times higher urinary N concentrations than the cows on the fodder beet treatment (P<0.001).

Table 4.1 Total mean urine N concentrations (g L^{-1}) on fodder beet and kale treatments.

	Fodder beet	Kale	SEM	P-value
Urine N	4.02	4.89	0.238	<0.001

4.3 Urine Harness Results

Figure 4.2 is an example of the urinary behaviour of a cow grazed on the kale diet. The figure illustrates the variability in urination.

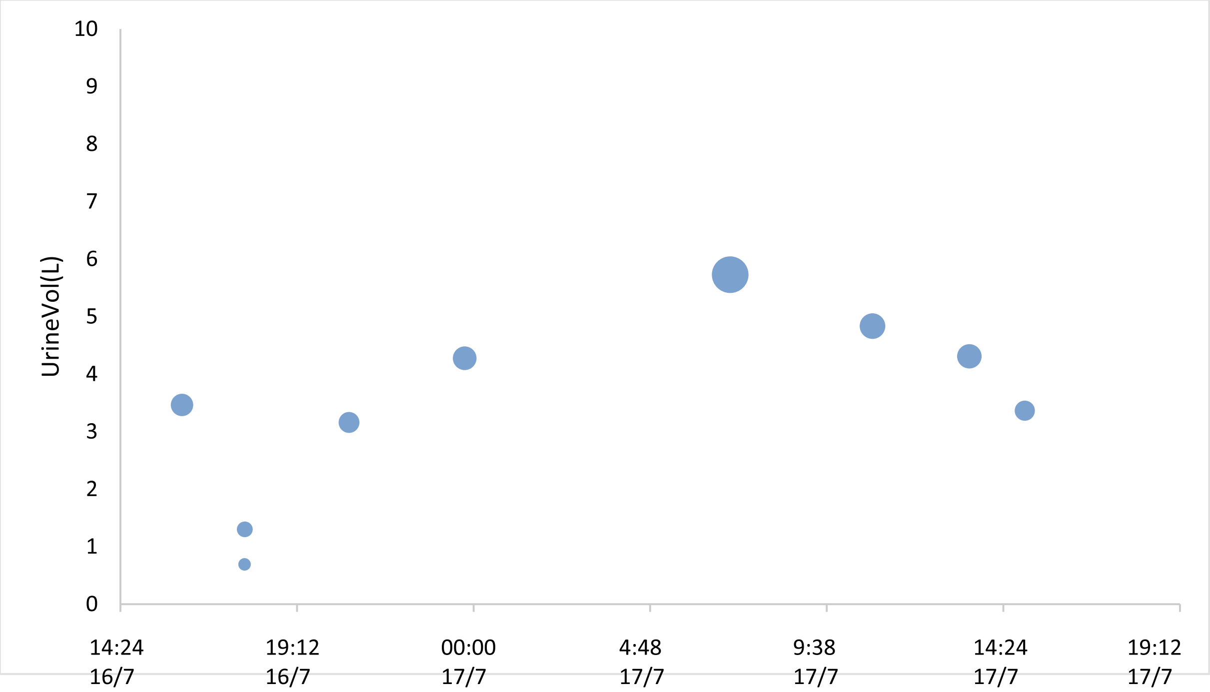

Figure 4.2 Date, time and size of urination events for Cow 9 on kale treatment

4.3.1 Urine frequency and volumes

Averaged results of the three urine harness trials are shown in Table 4.2. Cows on the fodder beet treatment averaged 8.2 ±2.25 urinations in 24 hours, ranging from 3 to 12 urinations. Cows on the kale treatment averaged 12.3 ± 2.25, with a range of 6 to 21 urination events, but the difference was not statistically significant ($P>0.05$). Similarly, average volume of urination events did not differ between the two treatments ($P>0.05$), averaging around 2.39L.

Total daily urine volume differed significantly between the treatment groups ($P<0.05$), cows on the kale diet urinated daily volumes that were 1.7 times more daily volume than volumes on the fodder beet diet (29.91 ± 4.7 versus 17.97 ± 1.9L/ 24 hours respectively).

There was no re-ranking of urine variables when values were adjusted for liveweight.

Table 4.2 Average frequency (No. per 24 hours), total daily volume (L/ 24 hours), average urination volumes (L), average daily urination volume and average urination event volumes per kilogram of liveweight of dairy cow urinations on fodder beet and kale treatments.

	Fodder beet	Kale	SEM	P-value
Frequency of Urinations (per 24h)	8.19	12.3	2.25	0.098
Frequency (Urinations/ hr)	0.34	0.51	0.09	0.098
Total Urine Volume (L/24h)	17.97	29.91	4.56	0.026
Average Urination Volume (L)	2.31	2.46	0.29	0.607
Average Liveweight (kg)	498	493.2		
Urination Volume (ml/ kg LW)	4.66	4.98	0.57	0.583
Daily Urine Volume (ml/ kg LW)	36.17	61.28	10.24	0.034

4.4 Urine Patch Results

4.4.1 Urine Patch Calibrations

Calibration curves that were generated to quantify the relationship between the volume of urine deposited and the area of the urine patch produced are shown in Figures 4.3 and 4.4. Both calibration curves produced linear equations showing strong regression relationships ($R^2 > 0.8$) between the volume deposited and the area of the patch.

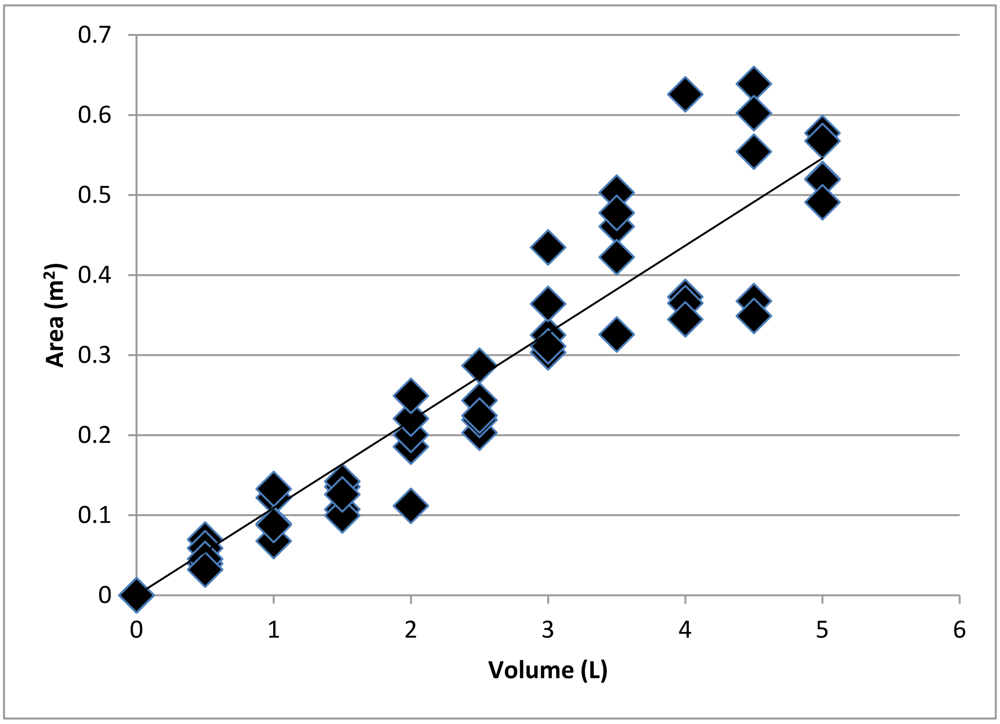

Figure 4.3 Calibration curve depicting the relationship between the volume of urine and the area of the urine patch produced on the fodder beet treatment. The equation for the linear relationship is Area = 0.1092 x volume. $R^2 = 0.888$

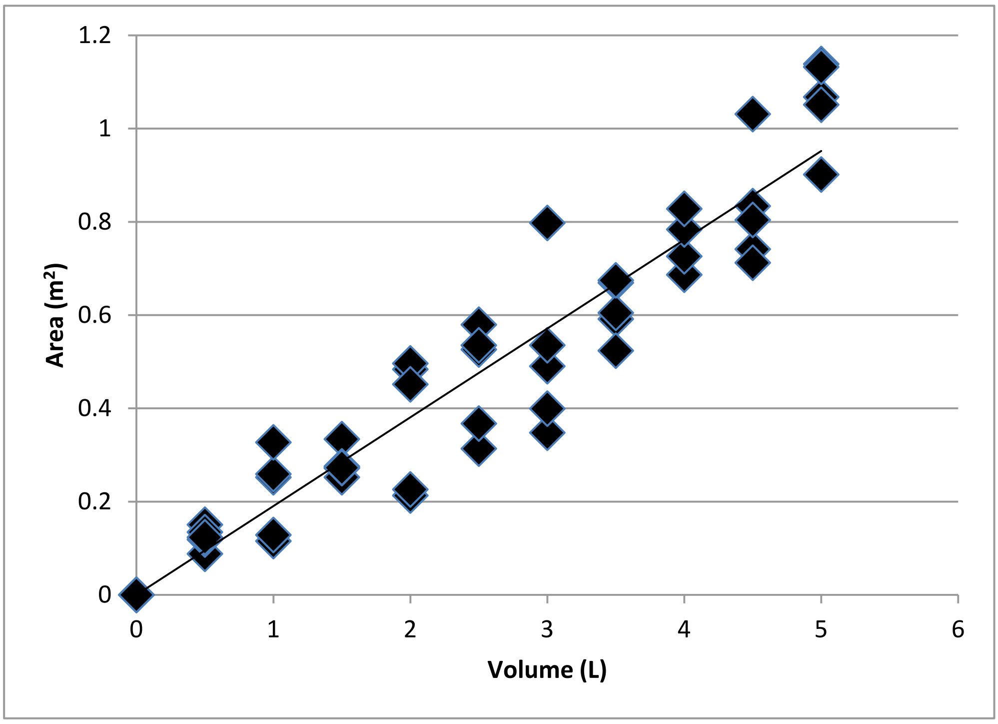

Figure 4.4 Calibration curve depicting the relationship between the volume of urine and the area of the urine patch produced on the kale treatment. The equation for the linear relationship is Area = 0.1901 x volume. $R^2 = 0.8882$

4.4.2 Urine Patch Areas and Volumes

Results of the average size (m^2) of all urine patch observations collected are shown in Table 4.3. On average, the urine patches produced on the kale treatment were found to be 1.3 times larger than those on the fodder beet treatment ($P<0.01$).

Table 4.3 Average area (m^2) of urine patches deposited by dairy cows on either fodder beet or kale treatments. Photographs taken between 1 and 4pm.

	Fodder beet	Kale
Area	0.1935	0.2442
S.D	0.1262	0.092
S.E	0.0138	0.0099
R^2	0.888	0.8882

4.5 Urine Harnesses Results and Calibration Curve Equations

Average volume of the urination events from the urine patch area data was calculated using equations produced from the calibration curves; results are shown in Table 4.4. The average volume of fodder beet urine patches was 1.4 times larger than the volume of the kale patches. Both values are less than their respective counterparts of average urination volumes shown in Table 4.2 (2.31 and 2.46L for fodder beet and kale respectively).

The average area of urine patches produced during the harness trials was calculated using calibration curve equations, results are also shown in Table 4.4. The average area of kale treatment patches was 1.9 times larger than the area of the fodder beet urine patches.

Table 4.4 Average volume (L) of urinations based on areas from urine patch observations, and average areas (m^2) of urine patch produced based on the average urination volumes, calculated using the calibration curve equations.

	Fodder beet	Kale
Calculated Volume	1.77	1.28
Calculated Area	0.25	0.47

Average volume of urinations from the harness trial produced between 1 and 4pm are shown in Table 4.5. This time period was the same as when the urine patch wetted area observations were collected. Results show that urination events in this time period were less than the total average volumes of urination events. Urination volumes on the fodder beet were only 77% of the average harness volume (2.31L), and 83% for the kale treatment urinations (2.46L).

Table 4.5 Average volume of urinations produced between 1 and 4pm on fodder beet and kale treatments.

	Fodder beet	Kale
Volume	1.77	2.05

4.6 N Leaching Calculations

Calculations of the estimated leaching losses on both treatments are described in 4.6.1 and 4.6.2. Estimates show that leaching losses were 1.4 times larger on the fodder beet treatment than the kale treatment (77.82 vs. 53.79 kg ha^{-1} respectively).

4.6.1 Fodder beet

Urine N concentration = 4.02g N L^{-1} Average urination = 2.31L Average patch area = $0.25m^2$

Urine N load in average urination = 9.28g

Urine patch N load $= \frac{9.28g}{0.25m^2}$

$= 371.45\text{kg ha}^{-1}$

Leaching losses from urine patches = 371.45 kg ha^{-1} x 32.16%

= 119.46 kg ha^{-1}

Assuming 50 cows for 60 days, on 1ha area:

Urine patch coverage = 8.19 urinations per day x 0.25 m^2 per urination x 50 cows x 60 days = 6142.5 m^2; total grazing area 1ha, then urine patch coverage = 61.43% (38.57% no urine)

119.46 x 61.43% = 73.38 kg ha^{-1} 11.5 x 38.57% = 4.44 kg ha^{-1}

(73.38) + (4.44) = 77.82 kg ha^{-1}

4.6.2 Kale

Urine N concentration = 4.89g N L^{-1} Average urination = 2.46L Average patch area = 0.47m^2

Urine N load in average urination = 9.28g

Urine patch N load $= \frac{12.03g}{0.47m^2}$

= 255.95 kg ha^{-1}

Leaching losses from urine patches = 255.95 kg ha^{-1} x 33.53%

= 85.83 kg ha^{-1}

Assuming 50 cows for 60 days, on 3ha area:

Urine patch coverage = 12.3 urinations per day x 0.47 m^2 per urination x 50 cows x 60 days = 17343 m^2; total grazing area 3ha, then urine patch coverage = 57.81% (42.19% no urine)

85.83 x 57.81% = 49.62 kg ha^{-1} 9.9 x 42.19% = 4.18 kg ha^{-1}

(49.62) + (4.18)= 53.79 kg ha^{-1}

Chapter 5

Discussion

5.1 Urinary N concentration

Ruminant urinary N output is closely linked to dietary N content (refer to Section 2.5). Ruminants excrete between 75-95 % of the N they ingest (Eckard *et al.*, 2010) depending on the type and amount of the feed source. This is N wastage, driven by the significant gap between the N requirements of grazed forages and the requirements of the animal. Higher concentrations in dietary N intake result in greater urinary N concentrations (Castillo *et al.*, 2000). Results from this trial revealed higher urinary N concentration from kale diets (4.89 g N L^{-1}) compared with fodder beet (4.02 g N L^{-1}). These results are due to cows on kale consuming more N which was associated with a greater concentration of N in the kale herbage than fodder beet. The urinary N concentrations from the present study are greater on average than those reported by Edwards et al (In Press) over two winters where urinary N% was less than 4g N L^{-1}, although they found that the N concentration (% of DM) of fodder beet was consistently lower than kale crop (1.75 and 2.05% respectively) agreeing with our findings. Edwards *et al.* (In Press)found that kale treatment cows had urinary N values of 3.1 and 2.2 g N L^{-1}, while fodder beet treatment cows showed concentrations of 1.9 and 2.3 g N L^{-1} in 2012 and 2013 respectively. This higher level of urinary N concentration compared to previous trials may be due to higher N% in either the crops or supplements; this data was not collected for this trial so it cannot be compared to determine whether this may be the cause.

While the differences in crop N concentration may partially explain the higher urinary N in kale versus fodder beet, it is also important to consider the supplements that were fed to the livestock - barley straw, fed to the kale cows, has a much lower N% than the ryegrass baleage fed to the fodder beet cows (0.8 and 1.8% DM respectively (Jenkinson, Edwards, & Bryant, 2014)) Despite this however, the total daily N intake, when utilisation (87.2% kale and 99.6% fodder beet (Edwards *et al.*, In Press)) is taken into account, was still higher in the kale treatment than in the fodder beet (274g N/day vs. 247 g N/day). Therefore, the N concentration in urine was higher in the kale treatment cows due to higher daily N intakes.

There are no other studies where dairy cows were grazed on wintering forage crops to compare results with. Despite these higher N concentrations in the urine compared to previous years, the concentrations are still lower than those observed on pasture systems – Betteridge *et al.* (2013) found that the average load of N on pasture was 8.6g N L^{-1}, and Pacheco *et al.* (2010) found an average concentration of 5.7g N L^{-1} on pasture, varying depending on season and species. It is also worth noting that Betteridge *et al.* (2013) found that not all urination events had the same concentration, the most concentrated urine was excreted during the night.

5.2 Frequency and Volume of Urination Events

It is well documented that urination by dairy cows is highly variable, in both the frequency of events and also the daily and event volumes (Fuller, 1928; Betteridge *et al.*, 1986; Aland *et al.*, 2002; Oudshoorn, Kristensen, & Nadimi, 2008); this is not unexpected - biological systems are inherently variable. The results of this trial also showed high variation – frequency of urinations ranged between 3 and 21 urinations in 24 hours, and averaged 8.2 and 12.3 ±2.25 for fodder beet and kale respectively, equating to an average of 0.34 and 0.51 times per hour. These results are similar to other published data, Aland *et al.* (2002) found dairy cows urinated 8.95 times (range 5-18), while Fuller (1928) found the number to be 6.81 times (range 2 – 13). While both of these studies were conducted overseas and with housed cows, which may arguably mean different results; on a grazing system daily urinations were found to be 9.8 times per 24 hours (Castle, Foot, & Halley, 1950). Draganova, Betteridge, and Yule (2010) conducted a study in New Zealand on grazed dairy cows and found urination events occurred 0.54 times per hour per cow. These results suggest that while there was variation in the system, on average dairy cows frequency of urination occurs somewhere between 7 and 13 times per 24 hours.

The results of this trial concluded that there was no difference between the two treatments on the average volume of a urination event, finding this to be around 2.39L ± 0.29. This result is consistent with that of Betteridge *et al.* (2013) who found the average urination volume to be 2.1L for cross-bred dairy cows which were grazing pasture.

The results in this trial suggest there was a significant difference ($P<0.05$) between the two treatments groups for total daily urine volume. Kale treatment cows produced an average of 29.9L ± 4.56 per day, while fodder beet cows only produced 18.0L ± 4.56. This difference could be due to the N content of the forage crops, or due differences in the crops' dry matter (DM) content. Animals fed a high protein diet consume more water, and excrete more urine (Bannink *et al.*, 1999); Khelil-Arfa *et al.* (2012) found that the content of crude protein (CP) ingested in forages and concentrates was the principal factor affecting the volume of urine. Jenkinson *et al.* (2014), who conducted their trial using the exact same treatment groups as the present study in 2013, found that CP% in the kale diet was 13.4%, while only 10.9% in the fodder beet diet. Additionally, the kale crop was found to contain 11.9% dry matter (DM), while the fodder beet crop contained 14.0%. Cows grazing on the kale were therefore consuming more water from the crop than their counterparts on the fodder beet crop, this was calculated at 70.6kg water day^{-1} vs. 51.4 kg water day^{-1} using values from (Jenkinson *et al.*, 2014).The higher daily water intake from feed may have resulted in higher volumes of urine being excreted as the water balance within the cow maintained its equilibrium.

There is very little daily and urination volumes data available to compare these results with as most urination studies only include observational data; additionally the results from this trial are limited by the small size of cows sampled, there were 7 individual cows sampled on the fodder beet diet and only 5 on the kale diet. In one of the few trials that does include urination volume results, Betteridge *et al.* (1986) also used a urine sensor, meaning the animals did not require constant observation for data to be collected. They found that the average daily volume was 25.6L, similar to the 29.9L produced by the kale treatment cows, however, unlike this trial; Betteridge *et al.*'s study was conducted on pasture-grazing steers. Grazed pasture has higher DM and CP% values (19.0 and 16.0%, respectively (Litherland & Lambert, 2007)) than the winter forage crops used in this trial, which is likely what is causing daily urination volumes closer to the kale diet.

5.3 Spatial Coverage of Urine Patches

The results indicated that the urine patch areas were significantly larger ($P<0.01$) on the kale treatment than the fodder beet (0.24m^2 vs. 0.19m^2 respectively). There are a large number of variables which determine the volume and area of soil affected following a urination, they include soil surface microtopography, moisture content, vegetation cover, slope, wind and the presence of pores open to the soil surface (Williams & Haynes, 1994). It is likely that these factors are the reasons driving the results obtained in this trial. The difference in area between the two crops was probably due to differences in the soil surface microtopography – on fodder beet crops the bulb grows partly submerged in the soil – when the dairy cows graze it they pull the bulb out of the soil leaving a large deep 'crater', diameters of bulbs can be greater than 15cm, and 40-50% of the bulb is buried within the soil (DLFSeeds, 2013). If a cow urinates on or near the bulb crater, urine rapidly pools into the crater as the force of gravity acts upon it. In contrast, kale grows and is grazed above ground and the soil surface remains relatively flat and level after grazing, so urine is able to spread without being pulled into depressions. The fodder beet was sown at a rate of 10.4 plants per m^2, and crop utilisation was high (90.1% DM utilisation (Jenkinson *et al.*, 2014)), so the area of the ground covered in 'craters' is significant.

The results obtained are contradictory to results from 2013 on these same two treatments; fodder beet was found to have urine patch areas of 0.57m^2, and kale 0.28m^2 (K. Cameron, unpublished data). The difference between these two results may be due to differences in the sample sizes, leading to outlier results having a larger influence on the mean result. This present trial sampled over 80 urine patches on each treatment type, significantly larger than the sample size for the 2013 trial –

only 11 observations on kale and 13 on fodder beet (B. Malcolm, personal communication). Results from both datasets included measurements where the urine patches were very large (>$0.6m^2$), so it is possible that with the smaller sample size, the average area on fodder beet paddocks was over estimated. Additionally, the individuals collecting the observations differed in both trials, and while the basic protocol for collecting the observation remained the same, ultimately outlining the urine patch comes down to the judgement of the observer.

Research into the spatial coverage of dairy cow urine patches is also scarce – the only other literature available is that of Moir *et al.* (2011), who found that urine patches ranged from 0.34 to 0.40 m^2 in area, with a 4-year mean of $0.37m^2$ ± 0.009. This is nearly twice as large as the areas found in the results of this trial, however, Moir *et al.* (2011) differ in their study in two key ways; firstly, their study was conducted on pasture - which differs from the two wintering systems used in this trial, and secondly the method of area measurement differs. As is previously mentioned, vegetation cover affects the area of soil that will be affected by a urination – post-grazing pastures still have a plant residual which mostly covers the soil surface, in both wintering systems there is barely any crop residual remaining, as is illustrated in Plate 3.5. Additionally, while the patch area in this trial was outlined immediately after the urination had occurred, in Moir *et al.* (2011), patch area was determined based on the visual identification of areas of lush, dense pasture growth, typical of a large pasture nitrogen response, weeks after the urine had been deposited. The area of pasture that responds to urine application may be actually larger than the initial wetted urine patch area due to lateral movement of nutrients by soil and roots (Dennis *et al.*, 2011).

5.4 Harness data versus urine patch calibration

The average urine patch areas obtained from the second part of the trial (0.19 and $0.24m^2$, fodder beet and kale respectively), are considerably lower than the results calculated based on the harness data and calibration equations (0.25 and $0.47m^2$ respectively). This difference can partially be explained by diurnal patterns in urination. Clark *et al.* (2011) found that the volume of urine more than doubled within an hour after feed was offered, similarly other studies have reported that the frequency and volume of urinations is higher when the cows are feeding, than when they are resting (Aland *et al.*, 2002; Hirata, Higashiyama, & Hasegawa, 2011). The cows were given their new crop breaks in the morning; by the time the urine patch observations were collected they had consumed the majority of their crop, and were primarily ruminating or resting. Jenkinson *et al.* (2014) reported that within 6 hours cows had consumed >75% of their daily intake, and Gregorini *et al.* (2009) suggest that up to 70% may be consumed in the first 3-4 hours after allocation.

Due to these temporal distribution patterns, the volume of urinations during the time period 1-4pm, when urine patch observations were collected, were lower than the average urination volumes collected by the harness devices, lower volumes would result in lower urine patch areas. For this reason, an analysis of harness data only during the 1 – 4pm time period was conducted. The results showed that the urination volumes during this period were lower than the average daily values, and aligned more closely with the volumes calculated based on the urine patch observations, particularly in the fodder beet treatment. Results of the urine patch area data calculated that the average volume of urinations were 1.77 and 1.28L for fodder beet and kale respectively, while the average urination event recorded by the harnesses was 2.31 and 2.46L respectively. When only recordings between 1-4pm were include these values reduced to 1.77 and 2.05L respectively.

It is also worth noting that the urine patch area data may be less reliable than the results of the harness data. The harness devices were calibrated sensors which removed any human sources of error; on the contrary, urine patch area measurements were visual observations where the outline of the patch was determined based on the judgement of the individual collecting them. The accuracy of the calculated area is also highly dependent on the fact that the photograph needed to be taken exactly parallel to the soil surface. This means there may have been a greater degree of error or bias in the observational results.

5.5 Estimation of leaching losses

The final leaching loss calculations of this trial showed that the greatest leaching losses occurred on the fodder beet treatment – leaching 1.45 times the level of N per ha than the kale (77.8 vs. 53.8 kg N ha^{-1} year^{-1}). This result was unexpected, as had the estimate been calculated using only urinary N concentration and urine harness data, the reverse would have been concluded. Cow urine produced on the fodder beet treatment in this trial had lower urine N concentration (4.02g N L^{-1}), lower average urine volumes (2.31L) and lower frequency of urination (8.2 urinations per 24 hours) than the kale treatment (4.89 g N L^{-1}, 2.46L and 12.3 urinations respectively). The reason therefore, for the higher leaching losses on the fodder beet crop compared to the kale is twofold; firstly, average urine patch area was smaller, and secondly the stocking rate was significantly higher on the fodder beet leading to higher urine patch coverage of the paddock.

The smaller urine patch areas on the fodder beet treatment compared to kale (0.25 vs. 0.47m^2) lead to a greater N load, expressed as kg N ha^{-1}; one urine patch on the fodder beet was calculated to have an effective application rate of 371.4kg N ha^{-1}, while the rate was only 255.9kg N ha^{-1} on the kale treatment. Additionally, due to the higher DM yield of fodder beet and daily allowances, the stocking rate on the fodder beet was 3 times that of the kale treatment. This meant that despite the average urine patch area on fodder beet being nearly half the size of a kale urine patch, paddock coverage of urine patches per ha, after 60 days was higher on the fodder beet treatment than on the kale treatment (61.4% vs. 57.8% coverage) due to the grazing intensity.

Because NO_3^- leaching is affected by a number of different factors (see Section 2.4), it is difficult to compare the predicted leaching losses with those estimated from other trials because key factors such as soil type and climate will differ significantly. Additionally, data on leaching losses, specifically from winter systems is, again, scarce. There is no published data that specifically compares losses on kale and fodder beet wintering systems, however Monaghan *et al.* (2007) modelled dairy wintering leaching at 55kg N ha^{-1} $year^{-1}$, noting that this was when the greatest losses occurred. Another study modelled wintering losses specifically on brassica crops at around 60 kg N ha^{-1} $year^{-1}$ (Chrystal *et al.*, 2012). These predicted leaching losses are similar to the 53.8 and 77.8 kg N ha^{-1} $year^{-1}$ calculated in this trial.

5.6 Practical Implications

One strategy that has been proposed for mitigating nitrate leaching losses is duration controlled grazing. Using lactating cows, Christensen *et al.* (2012) showed that duration controlled grazing reduced total N losses by 36%, while de Klein, Smith, and Monaghan (2006) recorded a 41% reduction in nitrate leaching losses. As has been established, Section 5.4, dairy cows do not require an entire 24 hours to consume their daily allowance of DM: within 6 hours cows had consumed >75% of their daily intake (Jenkinson *et al.*, 2014), and Gregorini *et al.* (2009) suggest that up to 70% may be consumed in the first 3-4 hours after allocation. While the livestock may not be eating for 24 hours, they are urinating over the entire 24 hour period (between 0.34 to 0.51 times per hour) (see Figure 4.2). Sections 5.1 and 5.3 describe results some studies have shown in the diurnal patterns of both urinary N concentration and urination volume. They suggest that while the volume of urinations is larger in the first hours after being given access to the daily allowance, urinary N concentration is higher in the evenings after they have finished grazing.

Therefore, because most of the forage crop is consumed within 6 hours, it may not be necessary for the livestock to be on the crop paddock for the entire 24 hour period; their removal from the paddock could result in lower urinary N deposition and thus lower N leaching losses. It is likely that applying this strategy to winter grazed forage crops would also result in reductions in leaching losses to these systems.

5.7 Conclusions

The purpose of this research dissertation was to develop and then use a urine harness capable of obtaining data on the variability of dairy cow urination events on kale and fodder beet winter grazing systems.

Results showed the urine N concentration was significantly higher in the kale treatment than the fodder beet treatment. This was because urinary N concentration is strongly related to dietary N intake. Results also showed high variability in urination frequency but there was no significant difference between either wintering system: average frequency equated to 0.43 urinations per hour, consistent with other published data. The results concluded there was no difference in the average volume of a urination event, finding this to be around 2.39L ± 0.29. There was a significant difference between the two treatments groups for total daily urine volume; kale diets produced an average of 29.9L per day, while fodder beet diets only produced 18.0L. Literature suggests this was likely due to the CP and DM content of the forage crops, as animals fed high protein diets consume more water, and excrete more urine.

Urine patch areas were significantly larger on the kale treatment than the fodder beet (0.24m^2 vs. 0.19m^2 respectively) due to differences in the soil surface microtopography. Estimates of leaching losses showed that the fodder beet treatment leached 1.45 times the level of N per ha than the kale (77.8 vs. 53.8 kg N ha^{-1} year^{-1}). Despite lower urine N, lower urine volumes and lower frequency of urinations on the treatment, the N load per urine patch was much higher due to the smaller urine patch areas, and the stocking rate was also significantly higher leading to higher urine patch coverage of the paddock.

Use of a duration controlled grazing system for either of these wintering systems may be used to reduce nitrate leaching losses (could be as high as 41%) without compromising DM intake as the majority (<75%) of the crops is consumed within 6 hours. If possible, in modelling systems used to predict leaching losses, the differences between these wintering systems should be taken into account.

5.8 Suggestions for further research

- Use of the urine harness to quantify urination information should continue, and be extended to include other livestock classes and physiological states, such as young stock and lactating dairy cows.

- The diurnal effect of N concentration in cow urine should be studied to further understand the processes surrounding urination. It would enable better understanding of how to mitigate leaching losses through duration controlled grazing, by determining when critical times for cows to be off the paddock are.

- The potential reductions in leaching losses on winter forage crop through duration controlled grazing should be trialled.

Appendix A

Consolidated Trial Data

	TRIAL ONE				Urination		
Cow Ear Tag No.		Hours	Number of Urinations	Frequency of Urinations (# per 24h)	Total Urine Volume (L)/24h	Average Urination Volume (L)	
11	Cow 1	22	8		25.234	3.154	K
154	Cow 2	48	20	10	14.4	1.44	K
49	Cow 3	48	24	14	30.18	2.156	K
46	Cow 4	48	17	8	21.919	2.739875	F
85	Cow 5	48	17	9	17.899	1.9887778	F
13	Cow 6	33	13	10	25.199	2.5199	F
	TRIAL TWO						
46	Cow 1	24	8	8	26.348	3.2935	F
65	Cow 2	ND					F
223	Cow 3	24	10	10	26.517	2.6517	F
9	Cow 4	24	10	10	23.524	2.3524	K
11	Cow 5	24	6	6	19.351	3.2251667	K
49	Cow 6	24	8	8	22.968	2.871	K
	TRIAL THREE						
46	Cow 1	24	9	9	12.54	1.3933333	F
49	Cow 2	24	7	7	4.092	0.5845714	K
14	Cow 3	24	6	6	15.38	2.5633333	F
68	Cow 4	24	10	10	18.219	1.8219	F
223	Cow 5	24	12	12	13.791	1.14925	F
65	Cow 6	24	3	3	8.687	2.8956667	F
154	Cow 7	24	11	11	23.981	2.1800909	K
9	Cow 8	24	12	12	27.245	2.2704167	K
11	Cow 9	24	8	8	31.131	3.891375	K
42	Cow 10	24	21	21	47.29	2.2519048	K

Fodder beet	Frequency of Urinations (# per 24h)	Total Urine Volume (L)/24h	Average Urination Volume (L)
Cow 4	8	21.919	2.739875
Cow 5	9	17.899	1.9887778
Cow 6	10	25.199	2.5199
Cow 1	8	26.348	3.2935
Cow 3	10	26.517	2.6517
Cow 1	9	12.54	1.3933333
Cow 3	6	15.38	2.5633333
Cow 4	10	18.219	1.8219
Cow 5	12	13.791	1.14925
Cow 6	3	8.687	2.8956667
Average	9.111111111	19.75689	2.2357299
Kale			
Cow 1		25.234	3.154
Cow 2	10	14.4	1.44
Cow 3	14	30.18	2.156
Cow 4	10	23.524	2.3524
Cow 5	6	19.351	3.2251667
Cow 6	8	22.968	2.871
Cow 2	7	4.092	0.5845714
Cow 7	11	23.981	2.1800909
Cow 8	12	27.245	2.2704167
Cow 9	8	31.131	3.891375
Cow 10	21	47.29	2.2519048
Average	11.75	27.58988	2.579

Cow	Frequency of Urinations (# per 24h)	Total Urine Volume (L)/24h	Average Urination Volume (L)	Average Urination Volume (mL)	LW	Event mL/kg LW	Total Urine Volume (mL)/24h	daily mL/kg LW
Fodder beet								
46	8.33	20.27	2.48	2475.57	469	5.28	20269	43.22
85	9	17.90	1.99	1988.78	465	4.28	17899	38.49
13	10	25.20	2.52	2519.90	502	5.02	25199	50.20
223	11	20.15	1.90	1900.48	504	3.77	20154	39.99
14	6	15.38	2.56	2563.33	553	4.64	15380	27.81
68	10	18.22	1.82	1821.90	518	3.52	18219	35.17
65	3	8.69	2.90	2895.67	475	6.10	8687	18.29
Average	8.19	17.97	2.31	2309.37	498	4.66	17972	36.17
Kale								
11	8	31.13	3.42	3423.60	520	6.58	31131	59.87
154	10.5	19.19	1.81	1810.05	497	3.64	19191	38.61
49	11	26.57	2.51	2513.50	487	5.16	26574	54.57
9	11	25.38	2.31	2311.41	502	4.60	25385	50.57
42	21	47.29	2.25	2251.90	460	4.90	47290	102.80
Average	12.3	29.91	2.46	2462.09	493	4.98	29914	61.28

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