INTERACTION EFFECTS OF LUCERNE (*Medicago sativa L.*) AND SUPPLEMENTS ON DRY MATTER INTAKE, MILK-SOLIDS YIELD, SUBSTITUTION RATE AND NITROGEN OUTPUT IN DAIRY PRODUCTION SYSTEMS

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Master of Agricultural Science

at
Lincoln University
by
Albert Muleke

Lincoln University, Canterbury, New Zealand

2013

Abstract of a thesis submitted in partial fulfilment of the
requirements for the Degree of Master of Agricultural Science.

INTERACTION EFFECTS OF LUCERNE (*Medicago sativa L.*) AND SUPPLEMENTS ON DRY MATTER INTAKE, MILK-SOLIDS YIELD, SUBSTITUTION RATE AND NITROGEN OUTPUT IN DAIRY PRODUCTION SYSTEMS

by

Albert Muleke

Abstract

Lucerne (*Medicago sativa* L.) is a major forage legume grown in approximately 45 million hectares worldwide. It is an excellent candidate for irrigation and can produce large quantities of quality herbage due to its high water use efficiency and deep root system. However, in New Zealand, the growth rate of lucerne is reduced in autumn/winter and early spring when temperature and solar radiation decrease. These periods of feed deficit usually provide opportunities for the use of supplementary feed to sustain high dry matter intake (DMI). Supplements are also offered as they may dilute the amount of nitrogen (N) eaten, thereby potentially reducing the quantity of N excreted in dairy cows grazing high N forages such as lucerne. Currently, little data exists on lucerne-supplement interactions in New Zealand dairy systems. This research study investigated the effect of feeding maize or grass silage supplements on dry matter intake (DMI), milk-solids (MS) yield, substitution rate (subR), grazing behaviour and N-output of dairy cows offered fresh lucerne in autumn. A field experiment was conducted at Lincoln University Research Dairy Farm (LURDF), Canterbury, New Zealand, between March and May, 2013. Three diets consisting of: 1. lucerne only; 2. lucerne plus maize silage, and 3. lucerne plus grass silage were allocated to 30 dairy cows in an incomplete randomised crossover design comprising of two periods of 15 days each. The silage supplementation, regardless of silage type, increased both total dry matter intake (DMI) and metabolisable energy intake - MEI (MJ ME/kg DM) compared to the lucerne only treatment, and reduced DM intake of lucerne herbage by up to 1.36 kg DM/cow/d ($P = 0.04$), resulting in a subR of 0.44 ± 0.03 kg DMI of lucerne/kg DMI silage and marginal milksolids response (MMR) of 0.02 ± 0.01 kg MS per kg DM silage intake. The type of silage had no effect on milk solids or milk and feed conversion efficiency ($P > 0.05$).

Generally, cows had low utilisation of lucerne herbage per hectare (44 ± 2%) and high utilisation of lucerne per cow (88.5 ± 3.8%), which had no effect on the rate of liveweight (LWT) gain and body condition (BC). The silage supplements reduced grazing time by up to 7 minutes ($P < 0.001$) during the morning grazing session of 4 hours, which could have been the effect of the supplements on physical fill of the rumen. The nitrogen use efficiency (NUE) averaged at 18.8 ± 0.5% for all cows in the three dietary treatments; however, feeding maize or grass silage did not improve the NUE. The supplements reduced the excretion of N via urine by 19 % and increased faecal N output by 8.7% and 4.0% for cows fed grass and maize silage respectively. The concentration of milk urea nitrogen (MUN) was highest in cows grazing lucerne only (15.85 ± 0.39 mmol/L; $P = 0.044$) compared to the supplemented cows (15.12 ± 0.39 mmol/L). The dietary treatments had no effect on urinary concentrations of creatinine, purine derivatives (PD) i.e. allantoin and uric acid, calculated PD index and the total microbial N supply. Conclusively, findings of this study show that feeding maize or grass silage to mid-late lactation dairy cows grazing lucerne in autumn significantly increased total DMI and MEI; however cow responses to the supplements in terms of milk-solids yield, subR, MMR, FCE, LWT gain and BCs were generally low. This therefore implies that supplementary maize or grass silage feeds can be used in autumn to sustain high DM and ME intakes, and enable substitution of the sown lucerne crop to increase herbage cover on the farm during periods of reduced growth in autumn/winter and early spring. The results also demonstrated the use of either maize or grass silage as a mitigation strategy to reduce nitrogen excretion through urine in dairy cows grazing autumn lucerne. Thus, based on these results, it would be appropriate to recommend the restriction of lucerne allowance when supplements are fed. This would improve the utilisation of lucerne per hectare, reduce the N-intake (particularly from lucerne herbage), and maintain milksolids production, milk yield and liveweight gain in mid lactation.

Keywords: Lucerne, Supplement, Dry matter intake, Substitution rate, Marginal milk response, Nitrogen use efficiency.

Acknowledgements

The study of Masters degree has been made possible through the award of a New Zealand Development Scholarship by the New Zealand Government through the Ministry of Foreign Affairs and Trade under NZAID programme. I am extremely grateful for this finacial support that enabled me to live and study in New Zealand up to completion of the study. I highly appreciate research funding from the Faculty of Agriculture and Life Sciences.

The completion of this thesis has been realized through conserted efforts from my supervisors, AGLS graduate team and the support from family.

I would like to express my deepest gratitude to my supervisors, Prof. Grant Edwards and Dr. Racheal Bryant for the excellent guidance, patience and providing me with an excellent atmosphere for doing research. Thanks a lot too for the great insight into the research topic, the constant advice on literature review, data analysis and discussion.

I would like to thank Dr. Andy Greer for willing to attend my research proposal seminar and evaluating the MSc. proposal. My appreciation also goes to Dr. Simon Hodge for the guidance on data analysis. Many thanks to Haldane Jan for being friendly and always offering to book meeting appointments with my supervisors.

I greatly appreciate the assistance of LURDF staff, namely Jeff Curtis, Helen Hague, Moniek van Rossum and Thomas Vazhathara, for the support that enabled me to sample accurately and effectively. I extend my sincere thanks to Rildo Moreira from Brazil, Aimi Nabilah Binti Hussein, Norfitri Bin Abdullah Sani, Mohamad Zaihan Bin Zailan and Nor Farhana Binti Ab Halim from Universersiti Putra, Malaysia, for your help which was timely and heartfelt; thank you also for the good trip around Christchurch, New Zealand.

I would like to extend my appreciation to fellow graduate students at the Department of Agricultural Sciences for their company, encouragements and willingness to help whenever needed. Big thanks to Geoff Smith for the guidance on calibration of lucerne. Many thanks also goes to Al – Marashdeh Omar for the assistance on loading maize silage barrels.

I am most grateful to staff members of Riddolls laboratory, namely; Diane Keaney , Jenny Zhao, Rosy Tung and Misato Iitaka for the training and assistance on proximate analysis using wet chemistry procedures for NDF, ADF, fats, protein, dry matter digestibility and ME. It was a fulfiling experience at the lab.

I would express my deep sense of gratitude for the affection and support from my parents; Prof. and Mrs Muleke, my two sisters; Everlyne and Christine, and brother; Price. Thank you all for the encouragement through best wishes. Thank you for just being there, to talk to and laugh with, and for the prayers too. And, to God be the glory forever and ever.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ANOVA	-	Analysis of variance
AAs	-	Amino acid(s)
ADF	-	Acid detergent fibre
BCs	-	Body condition score
BUN	-	Blood urea nitrogen
BW	-	Breeding worth
CP	-	Crude protein
CTs	-	Condensed tannin(s)
DM	-	Dry matter
DMD	-	Dry matter digestibility
DMI	-	Dry matter intake
ECM	-	Energy-corrected milk
EE	-	Ether extracts
FCE	-	Feed conversion efficiency
FMR	-	Forage mixed ration
FN	-	Faecal nitrogen
FV	-	Feeding value
HA	-	Herbage allowance
HF	-	Holstein Friesian
LWT	-	Live weight
ME	-	Metabolisable energy
MF	-	Milk fat
MJ ME/kg DM	-	Mega joules of metabolisable energy/kg of dry matter
MMR	-	Marginal milk response
MP	-	Milk protein
MS	-	Milk solids
MUN	-	Milk urea nitrogen
NDF	-	Neutral detergent fibre
NIRS	-	Near infrared spectroscopy
NUE	-	Nitrogen use efficiency
OM	-	Organic matter
PA	-	Pasture allowance
PD	-	Purine derivatives

PDIE	-	Protein digested in the small intestine at limited energy
PDIN	-	Protein digested in the small intestine at limited nitrogen
PDMI	-	Pasture dry matter intake
PPO	-	Polyphenol oxidase
PUE	-	Pasture utilization efficiency
PW	-	Production worth
r-DM	-	residual Dry matter
SIDDC	-	South Island Dairying Development Centre
SR	-	Stocking rate
SubR	-	Substitution rate
UN	-	urinary nitrogen
WSC	-	Water soluble carbohydrates

Chapter 1.
OUTLINE AND INTRODUCTION

1.1 Thesis Outline

This research thesis is divided into five chapters with Chapter 1 presenting a brief introduction to the use of lucerne and supplementary feeds in pasture-based dairy systems. The chapter also gives the aim and objectives of the thesis as well as research hypotheses. Chapter 2 reviews current literature on lucerne forage which include dry matter production and growth of lucerne in comparison to grass pasture and other legume forages in dairy systems. The chapter also evaluates previous research into the performance of animals fed lucerne with focus on DMI, grazing behaviour and effect of supplements on DMI, milk composition and milk-solids yield, substitution rate and marginal milk response and urinary-N output by grazing dairy cows. Chapter 3 on materials and methods, provides an overview of the experimental site at the Lincoln University Research Dairy Farm (LURDF) in New Zealand, and describes the experimental design. The chapter also describes the grazing management and outlines measurements taken on feed and cow parameters. Chapter 4 presents results on the measured parameters which consists data on lucerne, grass silage, maize silage and cow performance. Finally, Chapter 5 presents discussions, conclusions and recommendations for further research.

1.2 Introduction on the use of lucerne and supplements in dairy systems

Lucerne (syn. alfalfa, *Medicago sativa* L.) originated in the warm dry climate of Persia in central Asia before spreading to the Mediterranean and into North and South America (Michaud, Lehman, & Rumbaugh, 1988). It is now grown throughout the world for direct grazing or conservation as hay, silage and artificial dehydrated forage. In New Zealand, lucerne is a commonly grown pasture legume in dry land areas. Lucerne has the ability to produce large quantities of quality herbage. Average yields of up to 28 t DM/ha/yr. have been recorded under irrigated conditions on fertile soils at Lincoln University; (Brown & Moot, 2004; Hoglund, Dougherty, & Langer, 1974) and annual yields in excess of 18 t DM/ha can be obtained when water in non-limiting (Brown & Moot, 2004; Douglas, 1986). Irrigated dairy pastures based on ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) at Lincoln University's Dairy Farm have reported an average of 17 t DM/ha/yr. (SIDDC, 2010). In most cases lucerne has higher dry matter (DM) production over the comparative pasture, and can produce up to 25% more DM than pasture under irrigation.

However, when a 100% lucerne grazing system is compared with a 100% grass-pasture system, the inadequate seasonal feed distribution of lucerne becomes obvious (Mace, 1982). In New Zealand, lucerne lacks growth in autumn/winter and early spring (Mills, Smith, Lucas, & Moot, 2008), whereas the availability and quality of ryegrass pastures decrease during the summer-autumn months (Clark, 1995). Reliance on these low cost pastures (either ryegrass, lucerne or a mixture of both) for dairy farming often means milk production on New Zealand dairy farms drops sharply during these periods of pasture deficit (Clark, 1995). In the North Island, for example, the decline in milksolids production can be as high as 19% per month compared with a theoretical decline of 7% per month based on a normal calving spread and dairy cow physiology (Woodward, Chaves, Waghorn, & Laboyrie, 2002a). Developments in dairy farm management, such as irrigation, supplementary feed use and strategic nitrogen fertiliser to increase pasture growth have considerably increased milk production and lactation lengths, and flattened the lactation curve slightly (Woodward *et al.*, 2002a).

In the pasture-based dairy systems of New Zealand, autumn has been promoted as the most economical season to feed supplements (Woodward *et al.*, 2002a), as this may sustain intakes (White, 1982) and enable substitution of the sown crop to increase herbage cover on the farm going into winter. But often, the efficiency of converting feed to milk-solids and the subsequent marginal milk-solids response to supplements tend to be low in autumn compared to spring (Stockdale & Trigg, 1989), thus supplements are usually offered to improve the whole system response i.e. as pasture growth slows, extra feed can be used to extend the round and protect the residual, effectively growing more pasture and allowing more herbage to be harvested over a longer period, thereby increasing days in milk and milk production per cow (de Klerk, 2012). Supplements may also prevent bloat (Basigalup & Ustarroz, 2007; Bretschneider, Peralta, Santini, Fay, & Faverin, 2007). For instance, overseas in South American systems where lucerne has been used widely both as pure sward and within pasture mixes, some level of supplementation with conserved forages and/or concentrates are used to increase individual cow productivity under high pasture utilization efficiency (PUE) (Basigalup & Ustarroz, 2007).

Research studies also indicate that feeding low-N supplements can dilute the amount of consumed nitrogen (N), thereby reducing total N intake and, potentially, the quantity of urinary N (UN) excreted in dairy cows (Bargo, Muller, Kolver, & Delahoy, 2003; Castillo, Kebreab, Beever, & France, 2000; Poppi & McLennan, 1995). Thus, the N efficiency in pastoral systems can be improved by feeding low-N supplements rather than increasing energy intake (Ledgard, de Klein, Crush, & Thorrold, 2000; Valk, 1994). In a simulated study, Gregorini, Beukes, Bryant, and Romera (2010), reported that incorporating low-N maize silage supplement in ryegrass pasture diet of high producing dairy cattle was effective in reducing rumen ammonia nitrogen (R-NH3-N) and UN at the beginning of lactation, and slightly decreased the annual average UN: FN (faecal-N) ratio. Similarly, Dijkstra *et al.* (2009), reported reduction in urinary-N by feeding maize silage.

However, research data on direct feeding of lucerne to dairy cows under grazing conditions is currently limited when compared to ryegrass/white clover pasture in New Zealand (Moot, 2009). This study aimed to determine the effects of feeding maize and grass silage supplements on DM intake, milk solids yield, substitution rate and N-output of dairy cows grazing autumn lucerne in Canterbury, New Zealand.

1.3 Aim and objectives

Opportunity exists to develop grazing systems for the dairy industry based on lucerne monocultures or with grass mixtures in New Zealand. However, research data on feeding lucerne under grazing conditions in New Zealand is currently limited compared to ryegrass/white clover pasture (Moot, 2009). The aim of the research described in this thesis was to investigate the effects of supplements on DM intake, milk solids yield, substitution rate (SubR) and N-output of dairy cows grazing lucerne in autumn. The specific objectives were:

1. To quantify apparent dry matter intake, milk production and composition, urinary and faecal-N excretion for dairy cows grazing lucerne and supplemented with maize or grass silage in autumn.
2. To determine the substitution rate and marginal milk response for dairy cows fed fresh lucerne and supplemented with maize and grass silages.

1.4 Research Hypotheses

Previous studies with ryegrass pasture have shown that, when supplements are consumed by grazing cows, DM intake of pasture is usually reduced (Homes & Roche, 2007; Kellaway & Porta, 1993). An effect referred to as substitution, because supplement is substituting for pasture. Substitution can also result from a reduction in grazing time (Bargo *et al.*, 2003). A negative relationship exists between substitution rate and milk response to extra feed. Lower substitution rates are associated with higher total DM intake and consequently higher milk response to supplements (Bargo *et al.*, 2003; McEvoy *et al.*, 2008). Average immediate/marginal milk responses of 0.88 kg of energy-corrected milk (ECM) per kg of supplement and an average substitution rate of 0.36 have been calculated from experiments based on ryegrass/white clover pasture (Baudracco, Lopez-Villalobos, Holmes, & Macdonald, 2010). In this study, it was hypothesized that:

1. Maize and grass silage supplements will reduce apparent dry matter intake of lucerne, minimise urinary-N excretion, and increase N content in milk and faeces in dairy cows grazing lucerne in autumn.
2. Substitution rate and marginal milk response will result from feeding maize or grass silage to dairy cows grazing lucerne in autumn.

Chapter 2.

LITERATURE REVIEW

2.1 Brief Introduction

Lucerne (*Medicago sativa* L.), also known as alfalfa, is a temperate, perennial legume species originating from central Asia (Langer, 1973) and is a member of the *Fabaceae* family (Charlton & Stewart, 2000). It has an erect growth habit, making it suitable for grazing by sheep, cattle and deer (Charlton & Stewart, 2000). Its main advantage over traditional pasture is a taproot that enables the plant to draw water and nutrients from deep in the soil profile and ability to fix N. Lucerne has been promoted in New Zealand as the most suitable forage species for intensive dry land sheep pastures for over 100 years (Moot, Brown, Teixeira, & Pollock, 2003). On dairy farms, lucerne is mainly offered to lactating cows and replacement heifers as an alternative source of feed from late spring to late autumn as conserved hay or silage. The in *situ* grazing of lucerne in New Zealand dairy systems is currently uncommon, but in the late 1970s and early 1980s lucerne was used in the drier areas of the North Island following severe drought and grass grub infestation (Mace, 1982). This chapter reviews literature on the growth and DM yield of lucerne in comparison to grass pasture and other legume forages. The chapter also evaluates previous research into the performance of dairy cows grazing grass pasture and lucerne forage when offered supplements, with emphasis on DMI, milk composition and milk-solids yield, substitution rate and marginal milk response and urinary-N output.

2.2 Pasture growth and Dry matter yield

2.2.1 Lucerne growth

Knowledge of the growth and development of lucerne both within a regrowth cycle and across seasons is fundamental to understand recommendations for lucerne management. In many cases lucerne has higher DM production over the grass pastures, and its growth pattern suits incorporation with seasonal dairy farming. The pattern of lucerne growth and development within each regrowth cycle shows seasonal variation. Root and crown reserves of lucerne follow a cyclic pattern (Keoghan, 1991). They decrease during early vegetative regrowth and then increase with increasing plant maturity until full flowering (Figure 2.1). After growth is initiated, reserves decline until photosynthesis by the new leaf canopy is sufficient to exceed the needs of new shoot and root growth and maintenance (respiration). Typically plants will be about 15-20cm (6-8 inches) tall when food reserves reach their minimum and then begin to increase again. Note the reserves decline again if plants are left to produce seed because some are used to assist in seed development and also there will be demand for food reserves from the new crop of basal shoots elongating from the crown.

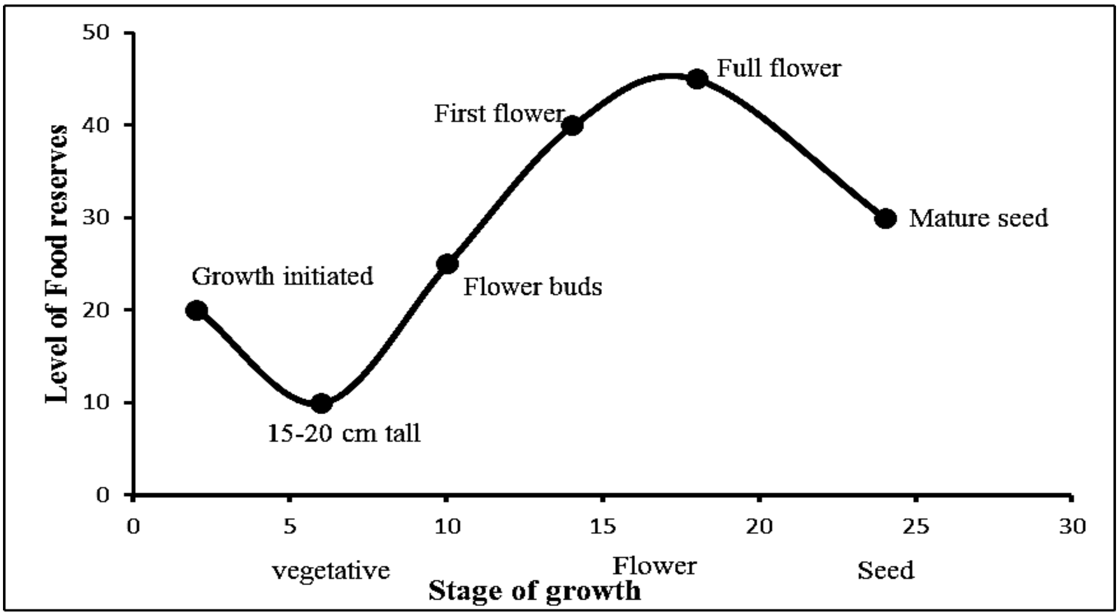


Figure 2.1: Changes in food reserves in lucerne roots and crowns from initiation of growth in spring to mature seed stage (Keoghan, 1991).

This growth pattern of lucerne is strongly influenced by temperature and solar radiation when water is not a limiting factor (Douglas, 1986). Growth rates are high in summer and low in winter (Brown, Moot, & Pollock, 2003). Specifically, shoot growth rates increase with increased temperature, but are higher in spring than in autumn at the same temperature (Figure 2.2).

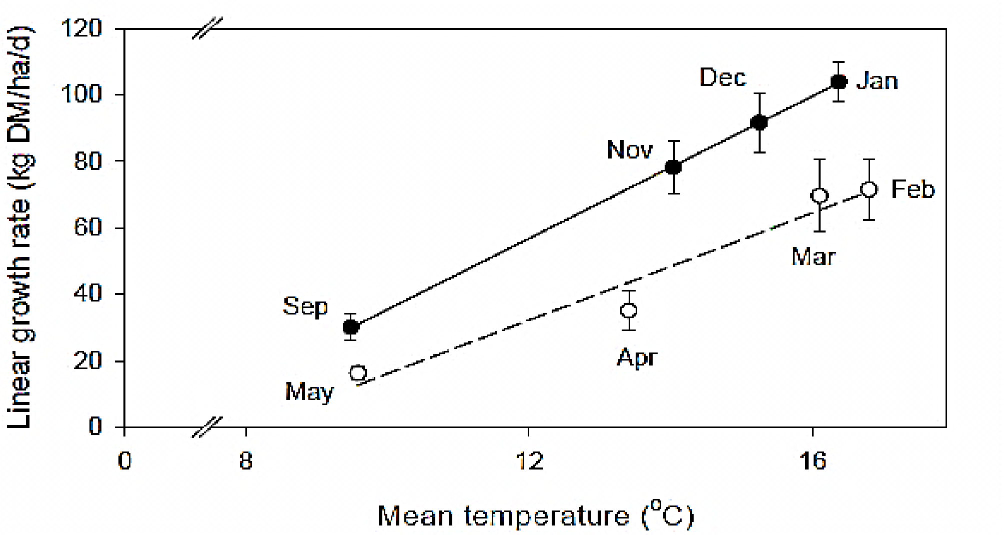


Figure 2.2: Linear growth rates of irrigated lucerne in relation to mean temperature at Lincoln University, Canterbury, New Zealand. Each point represents the mean from 5 years data and bars represent one standard error either side of the mean. Linear regressions fitted to data from September–January (●, $y = -71.5 + 10.7x$, $R^2 = 0.99$) and February–May (○, $y = - 64.7 + 8.0x$, R2 = 0.92) (Brown, Moot, Pollock, & Inch, 2000).

The difference in growth is caused by seasonal changes in the allocation of DM production between shoots and roots (Khaiti & Lemaire, 1992). In spring, roots lose weight as stored carbohydrates which are either lost in respiration or remobilised for the initiation of the new basal buds after defoliation (Kim, Ourry, Boucaud, & Lemaire, 1993). In contrast, autumn shoot growth is reduced because of increased assimilate partitioning to roots as plants replenish reserves for overwintering and spring regrowth (Hendershot & Volenec, 1993; Kim *et al.*, 1993); Kim *et al.*, 1991). Repeated grazing or cutting at immature (early) stages of growth (e.g. prior to the development of floral buds) or prolonged set stocking weakens the plants; they have reduced levels of root and crown reserves and reduced ability of the roots to seek and absorb water and nutrients. Rate of recovery is reduced, productivity declines and stand composition deteriorates (Keoghan, 1991). Thus, unlike many conventional pastures, lucerne requires careful grazing management to ensure maximum production and stand longevity. Rotational grazing throughout the year is recommended. This is primarily because lucerne grows from the tip of the stem rather than the base of the plant, and thus continuous grazing allows stock to remove new lucerne shoots and restricts the ability of the plant to regrow.

2.2.2 Lucerne dry matter production

Generally, lucerne has a clear average annual dry matter (DM) yield advantage over ryegrass / white clover in environments with low average annual rainfall. The yield potential of lucerne is 20 t DM/ha, depending on water availability and grazing management. For example, at Lincoln University, Canterbury (annual rainfall 460 to 660 mm) lucerne averaged 13.1 to 18.5 t DM/ha over five years, compared to cocksfoot / subterranean clover pasture at 9.9 to 12.9 t DM/ha and perennial ryegrass / white clover pasture at 8.0 to 12.9 t DM/ha (Mills *et al.*, 2008) (Figure 2.3).

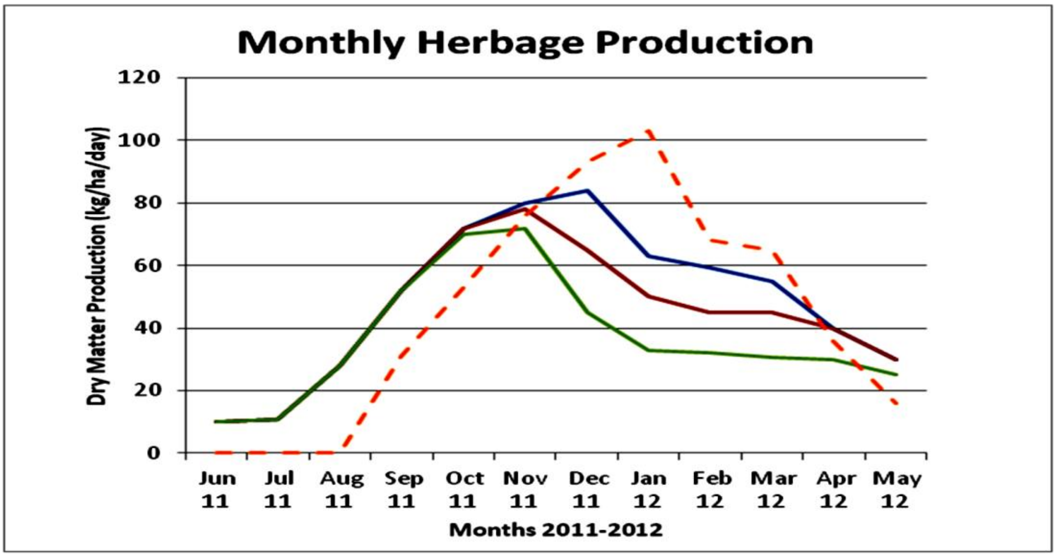


Figure 2.3: Monthly irrigated dry matter yield (kg/ha/day) on high fertility soil. Blue – Ryegrass -white clover mix monthly dry matter yield with a total annual yield of 17.8tDM/ha based on (Black, 2004). Red – Ryegrass- white clover mix monthly dry matter yield showing a 10% decrease in dry matter production (compared with blue) with a total annual yield of 16tDM/ha. Green – Ryegrass- white clover mix monthly dry matter yield showing a 25% decrease in dry matter production with a total annual yield of 13.4tDM/ha. Dotted orange: Lucerne monthly dry matter production under irrigation based on (Brown *et al.*, 2000).

Figure 2.3 describes the difference in seasonal production between lucerne and ryegrass. Most of the dry matter for lucerne was produced in the three spring months from mid-September to mid-January, while little growth occurred during winter. In contrast, pasture production was greater than lucerne in cool season (winter to early spring), although it was much less in spring, autumn and summer drought.

The superior nature of lucerne in comparison with other legume forages is well reported in literature. Herbage production, persistence, nutritive characteristics and utilisation of three perennial legume forages, chicory (*Cichorium intybus* L.), lucerne and red clover (*Trifolium pratense* L.) swards grown under irrigated and dry land conditions were compared over a 6-year period on a Wakanui silt loam soil in Canterbury, New Zealand (Brown, Moot, & Pollock, 2005) (Figure 2.4).

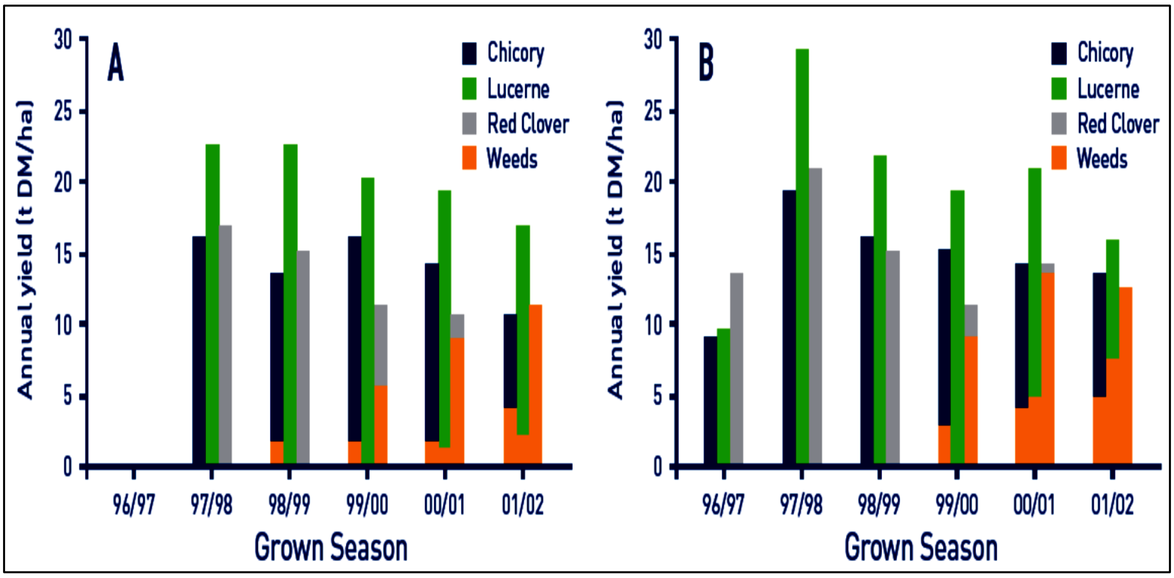


Figure 2.4 : Annual dry matter yields of (A) dry land or (B) irrigated monocultures of chicory, lucerne or red clover and weed yields. Forages were grown on a Wakanui silt loam at Lincoln University, Canterbury, New Zealand and were sown on 1/11/1996. Bars represent one standard error of the mean for comparison of species means within and between irrigation treatments. Upper bars are for total production and lower bars are for weed production (Brown *et al.*, 2005).

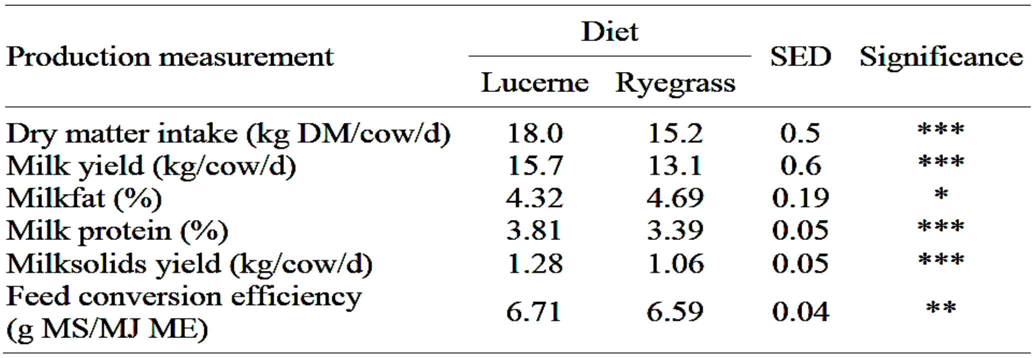
Under irrigated conditions, annual yields of lucerne (16-28 t DM/ha) were 30% greater than chicory or red clover (Brown *et al.*, 2005). Lucerne yielded 17.5-21 t DM/ha under dryland conditions, which was also 30-50% greater than chicory or red clover. The yield advantage of irrigated lucerne came from greater cool season growth, with 15 kg DM/ha per day higher growth rates in September and 10-30 kg DM/ ha per day higher growth rates from March to May (Brown *et al.*, 2005). Lucerne also had superior persistence, being 94% (dryland) and 55% (irrigated) of the botanical composition of swards in the sixth year, compared with 61% (dryland) and 55% (irrigated) for chicory and 0% for red clover. However, if lucerne is to be used in grazed dairy farms, a feed budget should consider the impact this will have on feed supply on the shoulders of the season in autumn and early spring, when the growth relative to ryegrass pasture is reduced. Supplementary feeds such as silage, hay, concentrates, green feeds or grass are usually offered during these feed deficits to sustain animal intakes (White, 1982). Basigalup and Ustarroz (2007), noted that if the objective is to increase individual cow productivity under high lucerne pasture utilization efficiency (PUE), some level of supplementation with conserved forages and/or concentrates must be used.

2.3 Animal performance

2.3.1 Lucerne DM intake

Intake of herbage has been identified as the main factor limiting milk yield of grazing cows (Dillon, 2007; Leaver, 1985). In New Zealand's pastoral dairying, nutritional needs of dairy cows are largely met by the supply of freshly grazed herbage from pasture, particularly from perennial ryegrass based pastures. Many reports show that when compared at the same digestibility, lucerne is eaten at slightly larger amounts than ryegrass. For example, in an indoor feeding experiment, Woodward, Waghorn, Attwood, and Li (2010), found that lucerne-fed cows always had a higher DMI than those fed ryegrass (Table 2.1), ranging from 12% higher on Day 4 to 23% on Day 10. A multitude of factors have been associated with this higher voluntary intake, including palatability and post-ingestion factors (Grovum & Chapman, 1988), that can be influenced by physical plant cues such as fibre content, plant and canopy structure, and chemical cues such as aroma, flavour, toxins, carbohydrate content, organic acid content.

Table 2:1: Dry matter intake, milk production, milk composition and feed conversion efficiency data from cows changed from ryegrass to lucerne on Day 4 or fed ryegrass throughout the experiment. The data here are the mean values for two treatments from Days 7 to 13 once milk production had stabilised. SED = Standard error of difference; DM = Dry matter; MS = Milksolids; ME = Metabolisable energy (Woodward *et al.*, 2010)



Production measurement	Diet		SED	Significance
	Lucerne	Ryegrass		
Dry matter intake (kg DM/cow/d)	18.0	15.2	0.5	***
Milk yield (kg/cow/d)	15.7	13.1	0.6	***
Milkfat (%)	4.32	4.69	0.19	*
Milk protein (%)	3.81	3.39	0.05	***
Milksolids yield (kg/cow/d)	1.28	1.06	0.05	***
Feed conversion efficiency (g MS/MJ ME)	6.71	6.59	0.04	**

However, other comparative studies have found no significant difference in DMI between cows offered lucerne and ryegrass. Smith, Bryant, and Edwards (2013), reported that the daily dry matter intakes based on pre- and post-grazing herbage mass, were similar for early lactation dairy cows fed perennial ryegrass only, grass plus lucerne and lucerne only, with consumption being in the range of 15.3, 15.0 and 15.2 ± 0.26 kg DM/cow/d respectively ($P > 0.10$). The authors also observed that consumption of nutrients on lucerne pastures was in favour of leaf components, due to the large reduction in leaf to stem ratio after grazing compared with pre-grazing. Before grazing lucerne leaf accounted for 55 ± 0.16% of the lucerne plant, furthermore, after grazing cows had removed 85% of the available leaf and only 6% of the upper stem.

2.3.2 Grazing behaviour of dairy cows

2.3.2.1 Grazing intake by dairy cows

Pasture DMI of grazing cows can be expressed as the product of grazing time (min/d), bite rate (bites/min), and bite mass (g DM/bite) (Hodgson & Brookes, 1999; Rook, 2000). Few grazing behaviour studies have been conducted with high producing dairy cows grazing lucerne under practical or production conditions, probably because of methodological limitations. Bite mass can be measured directly by using esophageally fistulated animals or indirectly dividing pasture DMI by the total bites/d (Forbes, 1988; Rook, 2000). Because use of esophageally fistulated animals is expensive and may compromise animal welfare and normal behaviour, bite mass is often calculated indirectly (Rook, 2000). Both biting rate and grazing time can be measured visually or automatically (Forbes, 1988). Visual estimation of biting rate requires the recording of head movements and sound associated with pasture prehension. Automatic methods for biting rate are based on recording jaw and sometimes head movements. Visual grazing time measurements are based on recording grazing activity at different intervals (e.g., 5 to 10 min), with the disadvantages of being labour intensive and limited by daylight (Rook, 2000). Traditionally, automatic grazing time measurements are conducted using vibracorders (Forbes, 1988). An automatic method for grazing behaviour estimation developed by Rutter, Champion, and Penning (1997), has several advantages such as fewer people required, less operator-associated errors, and more detailed behaviour information (Champion, Rutter, & Delagarde, 1998).

Among the three grazing behaviour variables, bite mass has the greatest influence on pasture DMI (Forbes, 1988; McGilloway & Mayne, 1996). Although bite mass is also affected by the animal's anatomy characteristics e.g., mouth; (Rook, 2000), it is principally determined by pasture-related characteristics (Hodgson & Brookes, 1999), such as pasture height (McGilloway, Cushnahan, Laidlaw, Mayne, & Kilpatrick, 1999; Phillips, 1993) and density (Rook, 2000). Pasture height is the major constraint on bite mass in temperate pastures, with the effect primarily on bite depth rather than on bite area (Rook, 2000). Dairy cows consistently remove around one-third of the height of pasture, regardless of pasture height (Wade, Peyraud, Lemaire, & Comeron, 1989). Bite mass decreases with a reduction in pasture height both in unsupplemented (Gibb, Huckle, Nuthall, & Rook, 1997; McGilloway *et al.*, 1999) and supplemented (Rook, Huckle, & Penning, 1994) dairy cows.

In many grazing behaviour studies, pasture height is expressed as sward surface height, which refers to the height of the top surface of the leaf canopy on an undisturbed sward (Hodgson & Brookes, 1999). Gibb *et al.* (1997), reported that for dairy cows continuously grazing ryegrass, bite mass decreased from 0.31 g OM/bite at 7 or 9 cm to 0.23 g OM/bite at 5 cm, whereas neither biting rate (76 bites/min) nor grazing time (604 min/d) were affected by sward surface height. McGilloway *et al.* (1999), found that bite mass decreased from 1.28 to 0.85 g DM/bite in one experiment with reductions in sward surface height (from 21 to 7 cm) and from 1 to 0.66 g DM/bite in a second experiment with reductions in sward surface height (from 11 to 6 cm), while biting rate was not affected (56 bites/min in experiment 1; 62 bites/min in experiment 2). In a third experiment, an interaction was found between sward surface height and density; bite mass was reduced with reductions in sward surface height more at low pasture density (from 1.02 to 0.47 g DM/bite) than at high pasture density (from 0.97 to 0.63 g DM/bite; (McGilloway *et al.*, 1999).

Grazing time and biting rate are influenced by animal-related characteristics such as genetic merit and milk production. Both grazing time and biting rate act as compensatory mechanisms to avoid reductions in pasture DMI when bite mass decreases. However, these compensatory mechanisms have a limit. The upper limit of grazing time to compensate for a reduction in bite mass is determined for the time required for other activities such as ruminating (Rook, 2000). Under poor pasture conditions (e.g., very short pasture), all three variables decline (Hodgson & Brookes, 1999). High genetic cows had higher grazing time and biting rate than low genetic cows supplemented with concentrate (Bao, Giller, & Kett, 1992). High genetic cows grazed a ryegrass pasture for longer time (218 vs. 204 min, measured visually for a period of 7 hrs.) and at a higher biting rate (64 vs. 61 bites/min) than low genetic cows. Two recent studies (Bargo, Muller, Delahoy, & Cassidy, 2002; Pulido & Leaver, 2001), reported that high producing cows had greater grazing time, number of bites per day and rate of intake than low producing cows.

2.3.2.2 Legume vs. Pasture grazing intake

A review by Popp, McCaughey, Thomas, and Cohen (1999), reported voluntary intake of legumes to be 28% greater than for equally digestible grasses (Minson, 1971). Higher intake of lucerne compared with grasses appears to be due to increased rate of digestion and increased rate of passage of the neutral detergent fibre (NDF) fraction (Hacker & Minson, 1981; Poppi, Minson, & Ternouth, 1981; Ulyatt, Lancashire, & Jones, 1977). The form, maturity and availability of herbage have all been shown to also affect voluntary intake (Chacon, Stobbs, & Sandland, 1976; Dougherty, Lauriault, Cornelius, & Bradley, 1989b; Stobbs, 1974). Dougherty, Lauriault, Cornelius, and Bradley (1989a), showed that cattle consumed fresh leafy lucerne herbage at a greater rate than wilted or stemmy herbage. Differences in consumption rates appeared to be related to bite size and biting rate, the former being controlled primarily by herbage availability and form (Hodgson, 1981; Stobbs, 1974). Factors that reduce bite size will also reduce intake rate (Dougherty, Lauriault, Cornelius, & Bradley, 1989c), which in turn results in increased grazing time and energy expenditure during grazing, which affects cattle performance negatively (Hancock & McMeekan, 1954; Hodgson, 1981; Popp, McCaughey, & Cohen, 1997; Stobbs, 1974). When herbage availability increases, intake rate also increases, even when pastures are of similar digestibility and protein content (Dougherty *et al.*, 1988). The higher intake rates observed for cattle grazing lucerne pastures can be attributed to the sward structure, which maximizes herbage intake per bite (Alder & Minson, 1963; Dougherty, Lauriault, Cornelius, & Bradley, 1987) and consequently reduces the time and effort spent in grazing (Popp *et al.*, 1997). With reduced energy expenditure for grazing leaves, more energy is available for production (ARC, 1980). Cattle grazing lucerne herbage have been reported to consume feed DM at a rate of 0.5 to 0.6% of their body weight per hour (Dougherty *et al.*, 1987). At equivalent herbage allowances, however, animals in rotationally stocked pastures tend to consume more nutrients per day than those in continuously stocked pastures (Popp *et al.*, 1997; Walker, Heitschmidt, De Moraes, Kothmann, & Dowhower, 1989; Walton, Martinez, & Bailey, 1981). Regardless of grazing strategy animal performance on lucerne is impacted to a greater degree by herbage availability through its effect on ingestive behaviour than by herbage quality (Dougherty *et al.*, 1988; Hart, 1972; Hart, Samuel, Test, & Smith, 1988; Heitschmidt, Dowhower, & Walker, 1987; Popp *et al.*, 1997; Ralphs, Kothmann, & Merrill, 1986; Walker *et al.*, 1989).

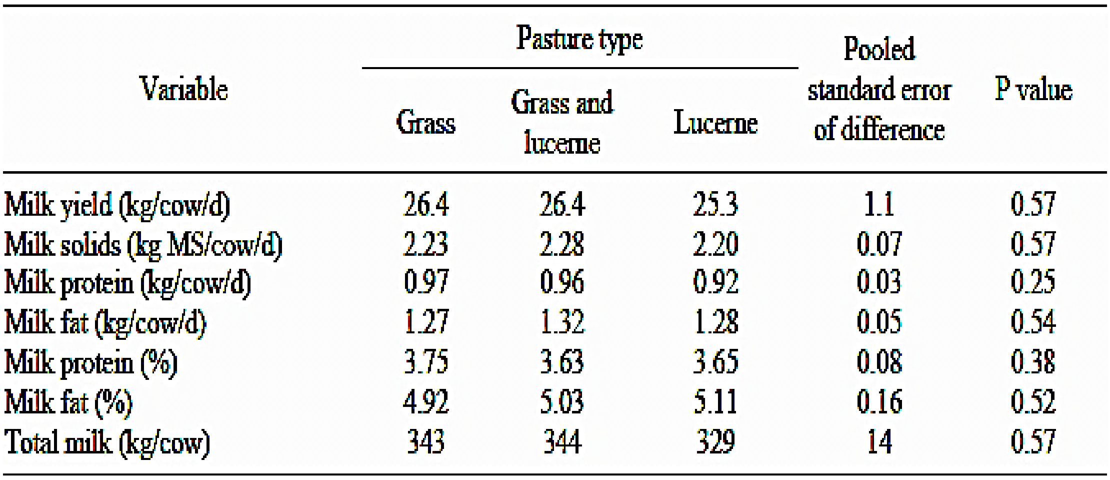
2.3.3 Milk production, composition and Milk-solids yield

The value of legumes for milk production has been attributed to higher intakes by cows and a higher nutritive value compared to ryegrass. However, there are fewer reports on milk production from cows grazing lucerne compared to grass pasture. Short-term experiments at DairyNZ’s Lye Farm, Hamilton, New Zealand showed that cows fed 95% lucerne produced 20% more milk per day than cows fed ryegrass-dominant pasture (Woodward *et al.*, 2010), which was attributed to higher forage quality and higher DM intakes. A recent dairy farmlet trial in the Waikato also evaluated productivity and profitability by comparing a farmlet based on ryegrass-white clover with a forage mixed ration (FMR) farmlet that included annual ryegrass, lotus, red and white clovers and lucerne (Woodward, Roach, MacDonald, & Siemlink, 2008). Milk solids production per cow was 9.5% higher on the FMR farmlet in 2006/2007 and 5.5% higher in 2007/2008, with most of this advantage in late summer-autumn when the FMR cows were producing 20% more milk. Woodward *et al.* (2008), concluded that including high nutritive value legume forages on-farm did have a positive response on MS production and profitability. Previous experiments where ryegrass was substituted with legumes have also increased MS production and the effects of feeding lucerne on milk composition mimicked changes reported when white clover, lotus and Sulla were fed (Harris, Auldist, Clark, & Jansen, 1998; Harris, Clark, & Laboyrie, 1998; Harris, Clark, Auldist, Waugh, & Laboyrie, 1997; Woodward, Auldist, Laboyrie, & Jansen, 1999; Woodward, Waghorn, Lassey, & Laboyrie, 2002b).

Mace (1982), reported that in general, lucerne is likely to increase milkfat production only on soil types where it consistently out-yields pasture by at least 30%, but the beneficial effects of lucerne are more obvious where it has a feed production advantage of 50% or more. Thomson and Lagan (1981), found that while lucerne produced 23% more dry matter than pasture, milkfat yield per cow was 21% less and milkfat per hectare was 12% less. Woodward *et al.* (2010), also showed significant differences in milk composition with reduced milk fat% compensated by an increase in milk protein (Table 2.1). Analysis of daily milk composition showed the change from ryegrass pasture to lucerne reduced milkfat and increased milk protein concentrations over three days, so from Day 7 lucerne fed cows had 8% lower milkfat and 13% higher milk protein concentrations.

Other component studies have however shown that milk production and composition from grazed lucerne is similar to grass when offered at same allowance, but urine has slightly higher nitrogen concentration due to more crude protein in diet compared to grass. In the UK, Limon Sanchez and Campling (1982) offered second growth lucerne for grazing by milking cows for 17 days in July and third growth for 10 days in August, 1980. The daily milk yield of the cows grazing lucerne was similar to that on ryegrass, and in this short trial, 60 to 75% of the lucerne was eaten at one grazing. In New Zealand, a field experiment established in spring by Smith *et al.* (2013) at Lincoln University Research Dairy Farm in September 2012, reported similar mean milk and MS yields between cows offered ryegrass and lucerne swards, averaging 26 kg/cow/d and 2.23 kg MS/cow/d respectively (Table 2.2). A validation study of whole–farm model (e-Dairy) conducted for lucerne-based dairy systems in Argentina and rye-grass-based dairy systems in New Zealand (Baudracco *et al.*, 2011), also showed similar patterns in DM intake, milk and MS yield in dairy cows at different stocking rate.

Table 2:2: Milk production variables and total two-week milk yield for dairy cows in early lactation grazing either, ryegrass/white clover pasture, lucerne in the morning followed by grass in the afternoon, or lucerne. SED = Standard error of the difference (Smith *et al.*, 2013).



Variable	Pasture type			Pooled standard error of difference	P value
	Grass	Grass and lucerne	Lucerne		
Milk yield (kg/cow/d)	26.4	26.4	25.3	1.1	0.57
Milk solids (kg MS/cow/d)	2.23	2.28	2.20	0.07	0.57
Milk protein (kg/cow/d)	0.97	0.96	0.92	0.03	0.25
Milk fat (kg/cow/d)	1.27	1.32	1.28	0.05	0.54
Milk protein (%)	3.75	3.63	3.65	0.08	0.38
Milk fat (%)	4.92	5.03	5.11	0.16	0.52
Total milk (kg/cow)	343	344	329	14	0.57

.

2.3.4 Animal supplementary feeding responses

2.3.4.1 The need for supplements in lucerne-based dairy systems

When a 100% lucerne grazing system is compared with a 100% pasture system, the inadequate seasonal feed distribution of lucerne becomes obvious (Mace, 1982). In New Zealand, a disadvantage of lucerne is its lack of autumn/winter and early spring growth (Mills *et al.*, 2008) and its quality declines rapidly as it matures, particularly in summer and autumn (Brown & Moot, 2004; Fletcher, 1976). To minimise the feed deficit during these periods of reduced growth, farmers could delay calving and/or feed supplements..

In addition to using supplementary feed to sustain animal performance when pasture supply is limited, there is also the possibility to explore the supplement option to mitigate the effects of higher rumen degradability of lucerne protein. Previous studies have reported higher N output in milk, dung and urine for dairy cows fed lucerne in comparison to pasture. Ledgard (2006), found urinary-N output to be higher (23 kg N/cow) for cows fed lucerne than those fed pasture (11 kg N/cow), cereal silage (5 kg N/cow) or maize silage (3 kg N/cow) (Table 2.3).

Table 2:3: Effect of feed source on N output in milk, dung and urine (kg N/cow/yr.) in absolute and relative terms (Ledgard, 2006)

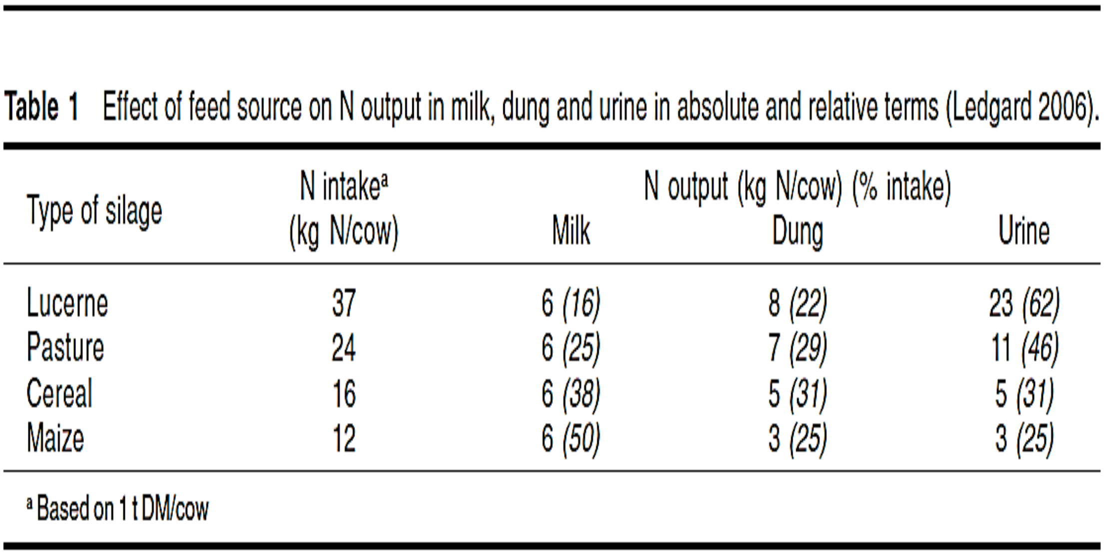


Table 1 Effect of feed source on N output in milk, dung and urine in absolute and relative terms (Ledgard 2006).

Type of silage	N intake[a] (kg N/cow)	N output (kg N/cow) (% intake) Milk	Dung	Urine
Lucerne	37	6 *(16)*	8 *(22)*	23 *(62)*
Pasture	24	6 *(25)*	7 *(29)*	11 *(46)*
Cereal	16	6 *(38)*	5 *(31)*	5 *(31)*
Maize	12	6 *(50)*	3 *(25)*	3 *(25)*

[a] Based on 1 t DM/cow

Van Vuuren and Mejis (1987), also examined the efficiency of cows fed the same dry matter levels of grass pasture or a 50:50 mix of pasture : maize silage diet and measured 40% greater conversion of dietary N into milk and 45% less N excreted urine from the pasture : maize silage diet. Many research studies investigating supplementary feeding of dairy cattle on temperate pasture have been reviewed with an attempt to provide predictive information regarding the response of dairy cows to supplementary feeds (Journet & Demarquilly, 1979; Kellaway & Porta, 1993; Leaver, 1985; Leaver, Campling, & Holmes, 1968; Mayne, 1991; Penno, 2002; Rattray, Brookes, & Nicol, 2007; Stockdale, 1997). Penno *et al.* (2006), demonstrated that the specific mixture of nutrients provided by supplements had little effect on the DM intake response to supplementary feeding. However, most of the data on the response of dairy cows to supplementary feed have been drawn from the interaction between grass-supplement in comparison to lucerne-supplement. This study aimed to quantify the responses to supplements by dairy cows grazing lucerne.

2.3.4.2 Response to supplements by dairy cows

Generally, when supplements are consumed by grazing cows, DM intake of grass pasture is reduced (Homes & Roche, 2007; Kellaway & Porta, 1993). An effect referred to as substitution, because supplement is substituting for pasture. The substitution rate (reduction in kg DM herbage intake per kg DM supplement consumed) increases as herbage allowance (HA) increases (Meijs & Hoekstra, 1984; Penno *et al.*, 2006). Substitution can also result from a reduction in grazing time (Bargo *et al.*, 2003). This could be considered an indirect cause, because the main cause is the reduction in the relative energy deficit of the cow (less hunger) as the feed allowance is increased by feeding supplements while maintaining the HA. The relative energy deficit is the amount of energy consumed by a cow relative to her demand. The deficit increases when energy demand is high, and/or when energy consumption is low (Grainger, 1990; Homes & Roche, 2007; Penno, Macdonald, & Holmes, 2001). Meijs and Hoekstra (1984), concluded that the effects of the factors influencing substitution rate may partly be related to the difference between the intake of nutrients from herbage and the nutrient requirement. Therefore, animal, sward, supplements and management factors that increase the relative energy deficit of the cow will decrease substitution rate.

Relative energy deficit between potential energy demand and actual energy supply is key driver of the response to supplements (Penno *et al.*, 2001). Milk response to supplementation is the increase in milk yield per kg DM supplement offered. A negative relationship exists between substitution rate and milk response to extra feed. Lower substitution rates are associated with higher total DM intake and consequently higher milk response to supplements (Bargo *et al.*, 2003; McEvoy *et al.*, 2008). Responses to supplementary feeds are highly variable. This is because they depend on a wide range of factors, involving the cows, feeds and management systems. If all the ME from extra feed consumed were converted into milk, 1 kg of MS would be produced, approximately, with an extra intake of 68 MJ of ME by a

500 kg LWT, New Zealand Holstein Friesian (HF) cow (Holmes *et al.*, 2002). Therefore, 1 kg DM intake as supplement (12 MJ ME/kg) would allow a theoretical milk response of 176 g milk-solid (MS) (2.3 litres of milk 7.7% MS). This is the maximum possible response from extra feed, assuming that all supplementary feed is consumed and all energy converted into milk (Homes & Roche, 2007). However, in practice, responses will be lower than the maximum possible, because the consumption of supplementary feed usually causes some decrease in pasture consumption (substitution) and some increase in LWT gain.

When the marginal and the carry-over effects of including supplements in a pastoral system are added, the total response to extra feed will almost always be smaller than the expected response (Holmes & Matthews, 2001). Average responses from 78 to 99 g MS/kg DM supplemented (with 12 MJ ME/kg DM) has been reported for whole-lactation experiments (Clark, 1993; Macdonald, 1999; Penno, 2002), which is around 1 kg milk. Kellaway and Porta (1993), reviewed experiments using supplementary feeds in Australia and concluded that when pasture was restricted, offering concentrates was likely to result in an immediate effect of 0.5 kg milk/kg concentrate fed (about 41 g MS/kg DM). However, Bargo *et al.* (2003), in a review of grazing experiments, reported that milk production increased linearly as the amount of concentrate increased from 1.8 to 10 kg DM/cow per day, with an immediate milk response of 1 kg milk/kg concentrate for high-yielding dairy cows. In agreement with these previous studies, Dillon (2007), reported that up until the early 1990s, average substitution rates published were around 0.6, resulting in an immediate response of approximately 0.4 to 0.6 kg of milk per kg of concentrate, with most of these studies carried out with low- to moderate-yielding cows (15 to 25 kg per cow per day). A lower substitution rate (0.40) and higher immediate milk response (0.92 kg of milk per kg of concentrate) were found in recent studies (Dillon, 2007).

Average immediate/marginal milk responses of 0.88 kg of energy-corrected milk (ECM) per kg of supplement and an average substitution rate of 0.36 were calculated from recent experiments (Baudracco *et al.*, 2010). The higher response to concentrate supplementation with higher genetic merit cows may be attributed to greater nutrient partition to milk production than with lower genetic merit cows (Dillon, 2007).

A review of studies evaluating the effect of supplementation on grazing behaviour of dairy cows by Bargo *et al.* (2003) showed that increasing the amount of supplement reduced grazing time but did not affect biting rate (Arriaga-Jordan & Holmes, 1986; Bargo *et al.*, 2002; Gibb, Huckle, & Nuthall, 2002; Kibon & Holmes, 1987; Rook *et al.*, 1994). Arriaga-Jordan and Holmes (1986), reported that grazing time was reduced by 11 minutes/kg of concentrate in continuous grazing and 8 min/kg of concentrate in rotational grazing, while biting rate was not affected by the amount of supplementation. Similarly, Rook *et al.* (1994), reported that concentrate supplementation, but not pasture height, reduced grazing time by 20 min/kg of concentrate, and bite mass decreased as pasture height decreased, while the supplementation amount had no effect on bite mass (Rook *et al.*, 1994). Amount but not type of energy supplement (cereal vs. beet pulp) reduced grazing time 8 to 12 min/kg of concentrate by dairy cows grazing ryegrass at two pasture heights (Kibon & Holmes, 1987). Biting rate was not affected by supplementation amount, type of supplement or pasture height, while bite mass was lower at the low pasture height (Kibon & Holmes, 1987). Recently, Sayers (1999) found that total bites per day and grazing time were higher when cows were supplemented with a fibre-based concentrate than when cows were supplemented with a starch-based concentrate, whereas bite mass was not affected. When the amount of concentrate was increased from 5 to 10 kg/d, total bites/d decreased from 22,023 to 16,933 and grazing time decreased 16 and 20 min/kg of fibre-based or starch-based concentrate, respectively (Sayers, 1999).

Bargo *et al.* (2002), reported that supplementation with 7.9 kg/d of a corn-based concentrate reduced grazing time by 75 min/d at low PA and by 104 min/d at high PA. Neither biting rate nor bite mass was affected by treatments. Gibb *et al.* (2002) reported that as the amount of concentrate supplementation increased from 1.2 to 6.0 kg/d, grazing time of dairy cows grazing a ryegrass pasture decreased numerically from 591 to 572 min/d. Supplementation with 2 kg/d of soybean meal did not change any grazing behaviour variables of dairy cows grazing a ryegrass pasture fertilized with 0 or 60 kg of N/ha (Delagarde, Peyraud, & Delaby, 1997). Bite mass was higher when cows grazed the fertilized pasture (Delagarde *et al.*, 1997). Sayers (1999), studied the effect of amount and CP content of concentrate supplementation on grazing behaviour of dairy cows on a ryegrass pasture. None of the grazing behaviour variables were affected by the CP content of the concentrate. Total bites per day and grazing time were reduced as the amount of concentrate was increased, but neither the bite mass nor the biting rate changed (Sayers, 1999).

2.3.5 Utilisation of dietary protein for grazed pasture

One of the major challenges associated with diets consisting of grazed pasture is the low efficiency of protein utilisation. This can be largely attributed to impaired rumen function due to (1) the relatively high concentration of soluble protein, and (2) the imbalance in the supplies of carbohydrate and protein (Beever & Reynolds, 1994; Lantinga & Groot, 1996). When supplies of readily available energy (mainly water-soluble carbohydrates) in the rumen are sufficiently high, amino acids taken up by the microbes can be incorporated into microbial protein. However, when the availability of water-soluble carbohydrates is relatively low, either amino acids or structural carbohydrates of the plant are used by rumen microbes for the bulk of their energy supply. These compounds are relatively slowly degradable and, as a result, there can be a lack of both balance and synchronisation of N and energy release in the rumen. This leads to ammonia accumulation in the rumen, which is absorbed across the rumen wall and subsequently converted into urea (Miller *et al.*, 2001; Nocek & Russell, 1988). This urea is mainly excreted through the urine and rapidly converted to ammonia, which is highly prone to volatilisation (Jarvis, Hatch, & Roberts, 1989), and to nitrates, which can either be used by crops or lost through leaching (Smith & Frost, 2000).

Forage legumes with good nutritional value when grazed as a sole diet e.g. lucerne (*Medicago sativa* L.), red clover (*Trifolium pratense*), chicory (*Cichorium intybus*), lotuses (Birdsfoot trefoil - *Lotus corniculatus*; Lotus – *Lotus pedunculatus*) and sulla (Burke, Waghorn, & Chaves, 2002), usually have higher protein content (especially in the leaf), which is rapidly degraded in the rumen so very little plant protein reaches the small intestine (Dhiman, Cadorniga, & Satter, 1993).Whilst the crude protein content of grass with moderate levels of nitrogen (N) fertiliser is often relatively close to animal requirements (130 to 170 g/kg DM), legumes often contain much higher levels (180 to 300 g/kg of DM), much in excess of animal requirements (Dewhurst, Delaby, Moloney, Boland, & Lewis, 2009). Mills and Moot (2010), showed lucerne herbage had N yields of up to 510 kg N/ha/yr which were higher than any of the grass based pasture species, including the sown species in the perennial ryegrass/white clover pasture which yielded only 151 kg N/ha in the same year. High yields of herbage N were also recorded for temperate forage legumes grown under irrigated conditions, for example; 250 – 500 kg N ha^{-1} for lucerne (Kleinschmidt, 1967; Simpson, 1976; Teakle, 1957) and 200 – 500 kg N ha^{-1} for white and red clovers (Kleinig, Noble, & Rixon, 1974; Richardson & Gallus, 1932; Simpson, 1976). Brown and Moot (2004), compared irrigated lucerne with monocultures of chicory and red clover, and found the utilised portion of the lucerne swards to provide 30% greater crude protein (CP) and metabolisable energy (ME) than the other species. The CP content was found to be 290 g/kg DM in the leaf while the ME was equal to 10.9 MJ/kg DM. The latter figure was comparable to 11.2 MJ/kg DM found by Mills and Moot (2010). Utilisation of lucerne remained between 72 and 80% over the five year period, with the exception of an average of 65% utilisation in year four, when snow caused significant lodging. The higher N content in herbage gives production advantages both in terms of increasing the amount of protein available for animal intake, and also higher growth rates and water use efficiency.

Increasing the CP content of dairy cow diets may result not only in greater milk production (Armentano, Bertics, & Riesterer, 1993; Wu & Satter, 2000), but also in increased concentrations of ruminal ammonia and blood urea N and consequently greater urinary N losses (Figure 2.5) (Armentano *et al.*, 1993; Castillo *et al.*, 2001b; Christensen, Lynch, Clark, & Yu, 1993).The high level of protein in legumes and extensive degradation during ensilage means that freshly grazed and ensiled legume contain high levels of quickly degradable N. This leads to inefficient utilisation (Dewhurst, Fisher, Tweed, & Wilkins, 2003) and particularly high urinary N output (Cohen, Stockdale, & Doyle, 2006; Dewhurst, Davies, & Kim, 2010). Lucerne has a higher supply of rumen degradable protein. The dietary proteins are hydrolysed in the rumen depending on their solubility and are hydrolysed to produce amino acids which are further deaminated to ammonia (Kirsopp, 2001). If in excess of dietary requirements or in the absence of enough energy, the ammonia may not all be converted to microbial protein. Because ammonia is toxic in the rumen liquor it is transported out via the bloodstream to the liver where it is converted to urea at an energy cost to animal and excreted. This in combination with the slow winter growth means that potential N losses could be greater than ryegrass/white clover mixtures.

An analysis of nitrogen balance experiments conducted on 91 diets and 580 dairy cows in different countries (Castillo *et al.*, 2000), revealed that the average efficiency of utilisation of N intake for milk protein production is 28%. The detailed measurements needed for these studies mean that they have usually been done with stall-fed cows, occasionally with cut grass. The available evidence suggests that problems of urinary N are greater with cows grazing pasture than when fed silages and concentrates or total mixed rations. Castillo *et al.* (2000), showed that faecal N averaged 21% of N intake. Urine was the main route for excretion of N in dairy cows, particularly when they consume over 400 g N/day- which is the case with high-quality pasture and forage legumes. A curvilinear relationship exists between urine N levels and N intakes (Figure 2.5) (Castillo *et al.*, 2000).

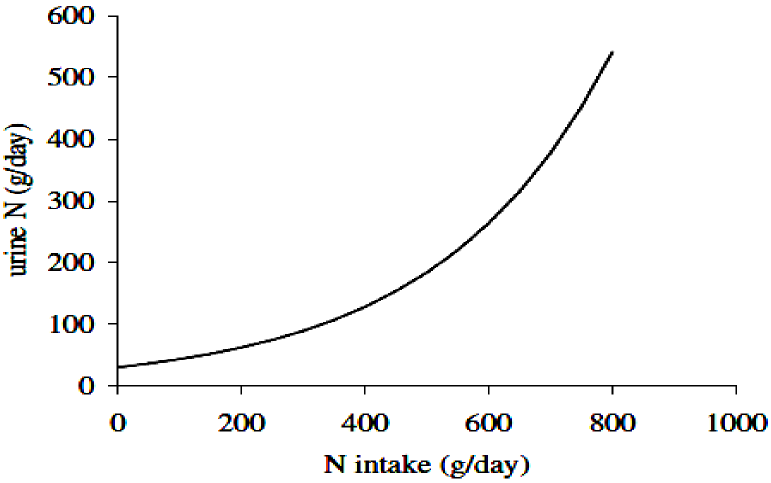


Figure 2.6: Relationship between N intake (g/day) and urinary N excretion (g/day) (Castillo *et al.*, 2000)

2.3.6 Improving NUE through pasture manipulation and supplemention

2.3.6.1 Pasture composition

The low utilisation of plant protein has often been associated with the imbalance between the rapid degradation of plant protein and the slow fermentation of most plant carbohydrate (fibre) in the rumen. This has been described as the rumen synchrony concept and has led to investigation of strategies to produce diets with slower protein degradation and faster carbohydrate availability (e.g. from increased water-soluble carbohydrate levels). A number of researchers have tried to improve rumen synchrony by identifying plant mechanisms, such as condensed tannins (CT) and polyphenol oxidase (PPO), which reduces protein degradation rates in the rumen. Unfortunately, the forages that contain these mechanisms- notably birdsfoot trefoil (CT) and red clover (PPO) - do not fit into dairy systems for other reasons e.g. the birdsfoot trefoil is not agronomically suited to most conditions and the action of PPO is inhibited when red clover is ensiled before feeding. Thus, in general, altering the characteristics of pasture proteins has not had major effects on nitrogen utilisation in practice (Dewhurst, 2006).

In contrast, there is much greater evidence for beneficial effects of increasing the supply of fermentable carbohydrates, particular when simultaneously reducing protein content. Ryegrass cultivars with a high water-soluble carbohydrate content led to a significant improvement in the utilisation of pasture N (from 30 to 37% in late lactation cows (Miller *et al.*, 2001). The success of high-sugar grasses in improving nitrogen utilisation may be as much to do with reduced crude protein content as it is with increased levels of water-soluble carbohydrates (Miller *et al.*, 2001). Reduced urinary N excretions are most likely to occur if ryegrasses can be produced with WSC: CP ratios of 0.7 or above (Edwards, Parsons, & Rasmussen, 2007), equivalent to CP:WSC of 1.3 or below (Pacheco, Burke, & Cosgrove, 2007). High protein and low water-soluble carbohydrate levels in autumn pasture are associated with low efficiency of utilisation of feed N (Beever, Terry, Cammell, & Wallace, 1978). Orr, Rutter, Penning, and Rook (2001), in the UK and Trevaskis, Fulkerson, and Nandra (2004), in Australia demonstrated improved intakes and a tendency for improved production when cows are given new pasture allocations in the afternoon, when water-soluble carbohydrates are higher. It is likely that this would also increase N-utilisation. Studies reviewed by Peyraud and Astigarraga (1998), all showed that reduced levels of N fertilisation caused only a small (i.e., 5% on average) reduction in the amount of non-ammonia N entering the intestine despite a much lower CP content and a slightly lower CP digestibility in less fertilised grass (Delagarde *et al.*, 1997; Peyraud, Astigarraga, & Faverdin, 1997; Van Vuuren, Krol-Kramer, Van der Lee, & Corbijn, 1992). This resulted in a decreased output in urinary N, whereas N excretion through dung was hardly affected (Peyraud & Astigarraga, 1998). However, when CP content falls below animal requirements, a reduction in both herbage intake and protein intake may result in decreased animal production (Minson, 1990; Peyraud & Astigarraga, 1998).

2.3.6.2 Level and type of supplement

In pasture-based systems, previous efforts to improve N capture have focused on improving energy supply to the rumen, with the objective of incorporating more ammonia into microbial protein and, thereby, increasing the AA flow to the small intestine (Kolver, Muller, Barry, & Penno, 1998b; Miller *et al.*, 2001; Moorby, Evans, Scollan, MacRae, & Theodorou, 2006; Sairanen, Khalili, Nousiainen, Ahvenjärvi, & Huhtanen, 2005). Studies and commercial practice in Europe and the US suggest possible solutions to the low efficiency of utilisation of pasture N through the use of low-protein supplements such as maize silage. North American dairying is based on the complementary characteristics of a high-protein legume (e.g. lucerne) and low-protein forage (maize silage) (Basigalup & Ustarroz, 2007). Similarly, UK work has shown good levels of milk production with low urinary N output by combining legume silages with maize silage (70% less urinary N per kg milk protein) (Dewhurst, Merry, & Davies, 2005). Lucerne contains high levels of rapidly degradable N and this is reflected in the difference between PDIN and PDIE values (INRA, 2007).

The supplementation with energy-rich concentrate which is required to overcome the relatively low energy concentration of legumes will reduce urinary N losses (Cohen *et al.*, 2006). When concentrate supplements were offered, the use of low-protein supplements rich in fermentable carbohydrates increased nitrogen efficiency (Keady, Mayne, & Marsden, 1998), whilst supplementation of pasture with soya bean meal (around 48% protein) led to a substantial increase in urinary N excretion (Delagarde *et al.*, 1997). However, even when low-protein supplements are used, the increased stocking rates achieved and importation of protein on to the farm make it likely that urinary N production per hectare will increase.

2.3.7 Potential Animal Health Risks

The danger of ruminants developing legume bloat (also known as pasture bloat) limits the greater use of legumes for grazing. Legumes such as red clover, white clover and lucerne can cause bloat (Majak, Hall, & McCaughey, 1995). When direct grazing lucerne some animal health issues can develop. These are most likely when the lucerne is lush in early spring (Moot, 2009). Bloat occurs due to a build-up of gas in the rumen and can lead to acute abdominal distension (Howarth *et al.*, 1991), with the rate of gas production being greater than the animal's ability to expel the gas from the rumen. Thompson, Brooke, Garland, Hall, and Majak (2000), showed that the early stages of lucerne growth were the most likely to cause bloat. This was associated with a higher CP concentration and a lower DM and fibre concentration. Majak *et al.* (1995), demonstrated an increase in the probability of bloat with increasing soluble protein concentration. The rapid release of soluble protein into ruminal fluid promotes the formation of a polysaccharide slime that traps the rumen gases (Clarke & Reid, 1974; Howarth *et al.*, 1991; Pinchak *et al.*, 2005). Other studies have demonstrated the importance of small particles e.g., chloroplast fragments from immature lucerne in the development of pasture bloat. The rumen bacteria attached to these particles have an abundance of carbohydrates, both internal (as storage granules) and external (as slime) which contribute to froth formation (Majak, McAllister, McCartney, Stanford, & Cheng, 2003). The small particles themselves also play an important role in froth formation as they become part of the slime matrix in which the gas bubbles get trapped (Majak *et al.*, 2003).

Bloat may be alleviated by restricting access to lucerne/alfalfa or by grazing cattle before they come in the sward. Supplementing cattle with roughage (e.g. hay or maize silage) ahead of grazing lucerne and/or graze lucerne alternatively with pasture grass. Some grazing management precautions that will also greatly reduce the risk of bloat (Pioneer, 2010), include avoiding grazing lucerne when it is fresh and lush (particularly in the spring and autumn following a break), and when the stand is immature. Mature stands are much safer. Hungry stock should not be put onto lucerne to prevent them from gorging on high quality feed which can cause bloating. When lucerne is lush, after rain, or during early spring, animals should be fed hay or allowed access to adjacent grass or weedy areas for roughage. Grazing cattle have access to anti-bloating agents (drenches, addition to the water supply, rumen bloat capsules or rumen bullets, spraying) especially in intensively grazed systems (Cook *et al.*, 2005).

At similar stage of growth lucerne has higher concentrations of the major mineral elements of nutritional importance compared to grass, white or red clover (Thom & Smith, 1980). One exception is that the concentration of sodium in lucerne is usually considerably lower than in grass. In New Zealand supplementation with sodium chloride of cattle and sheep grazing lucerne has been shown to have a marked beneficial effect on health and production (Jagusch, 1982).

2.3.8 Summary

Lucerne has the ability to produce large quantities of quality herbage. Average yields of up to 28 t DM/ha/yr. have been recorded under irrigated conditions on fertile soils in New Zealand. Despite this superior yield particularly when compared to ryegrass/white clover pastures and other forage legumes, lucerne lacks growth in autumn/winter and early spring when temperatures and photoperiods decrease. This in turn reduces both growth and development. These periods of feed deficit often provide opportunities for the use of supplementary feeds to sustain higher animal performance and conserve feed. Supplements are also offered as they may dilute the amount of nitrogen (N) eaten, thereby potentially reducing the quantity of N excreted in dairy cows grazing high N forages such as lucerne.

Most of the data on responses of dairy cows to supplementary feeding has been obtained mainly from grazing experiments with ryegrass dominant pastures in New Zealand. These data show that supplementary feeds when offered often reduce pasture DMI (i.e. substitution), due to the reduction in the relative energy deficit of the cow (less hunger). Substitution can also result from a reduction in grazing time, but this is an indirect cause. A negative relationship exists between substitution rate and milk response to extra feed. Average substitution rates published are around 0.6 (for early 1990s), resulting in immediate response of approximately 0.4 to 0.6 kg of milk per kg of concentrate, with most of these studies carried out with low- to moderate-yielding cows (15 to 25 kg per cow per day).

Studies show that DMI, milk production and composition from grazed lucerne is similar to grass when offered at same allowance, but urine has slightly higher nitrogen concentration due to more crude protein in diet compared to grass. Thus, opportunity exists to develop grazing systems for the dairy industry based on lucerne monocultures or with grass mixtures in New Zealand to complement grass pasture-based systems. However, research data on feeding supplements to improve dairy cow performance and reduce urinary N output under lucerne grazing conditions is currently limited compared to ryegrass/white clover pasture.

Chapter 3.

MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted at Lincoln University Research Dairy Farm (LURDF), Canterbury, New Zealand, between March and May, 2013 under the authority of Lincoln University Animal Ethics. The farm lies at latitude $43^{o}39'$ South and longitude $172^{o}27'$ East. With an altitude of approximately 18 meters above sea level (ASL). Mean annual maximum and minimum temperatures are 32 °C and 4 °C respectively and average annual rainfall is 666 mm (SIDDC, 2010). The experimental area was made up of 4 lucerne paddocks (labelled A2, A4, A5 and A7; Figure 3.1); each of 1.5 ha in size situated on a mix of Templeton silt loams and Paparua sandy loam soils. The 4 paddocks were drilled as pure swards at 10 kg seed/ha with cv. Force Four lucerne (*Medicago sativa* L.) sown on 15 February 2012 after a double spray (Glyphosate 3L/ha) summer fallow and full cultivation. Trifularin (2L/ha) was used as a pre-emergence herbicide to control early weed establishment. Gallant™ Ultra (300mls/ha) was applied to control annual grass weeds that established with the crop mainly *poa annua* and also 65 g of Preside™ was sprayed onto crop to control established broadleaf weeds. Selection of cows used in the experiment was done on 25th February, 2013 and data collection was done from 4th March to 24th April, 2013..

3.2 Experimental design and treatments

The experiment was an incomplete randomised crossover design consisting of three diet treatments and two periods, each of 15 days with an adaptation phase and washout period of 7 days each (Table 3.1). Experimental treatment diets were: 1. strip-grazed lucerne only offered at 15 kg DM/cow/day **(L)**; 2. lucerne (15 kg DM/cow/day) plus maize silage offered at 1.5 kg DM /cow/day in the morning (a.m.) and 1.5 kg DM/cow/day in the afternoon (p.m.) **(L+M)**; and 3. lucerne (15 kg DM/cow/day) plus grass silage offered at 1.5 kg DM/cow/day in the morning (a.m.) and 1.5 kg DM/cow/day in the afternoon (p.m.) **(L+G)**. Bloat was controlled using Rumensin® capsules (1 capsule per animal applied 7 days before transition period) and by using bloat oil in water troughs 40 ml/15L (20-40ml per head).

Dietary treatments were randomly allocated to a total of 30 Friesian and Jersey x Friesian, multiparous (450 kg LWT) cows in mid-lactation, from the LURDF herd. Treatment allocation of those 30 cows was then based on milk-solids production, liveweight and age (Table A. 5.1).

Table 3:1: Allocation of the three treatments of lucerne only (**L**), lucerne + maize silage (**L+M**) and fresh lucerne + grass silage (**L+G**) to 30 dairy cows in an incomplete randomised cross-over design.

Treatment	**Adaptation phase**	**Period 1 (15 days)**	**Wash out**	**Period 2 (15 days)**
Lucerne only **(L)**	7 days	10 cows	7 days	5 cows from L+M + 5 cows from L+G
Lucerne + maize silage **(L+M)**	7 days	10 cows	7 days	5 cows from L + 5 cows from L+G
Lucerne + grass silage **(L+G)**	7 days	10 cows	7 days	5 cows from L + 5 cows from L+M

Period 1: A total of 10 cows were randomly allocated to each of the three treatments as described above (**L**, **L+M** and **L+G**) to constitute 30 dairy cows. The cows were allowed an adaptation phase of 7 days and the diets offered for 15 days.

Period 2: The experiment was repeated for another 15 days after a 7 day washout period. Five cows were randomly selected from treatment **L** and allocated to treatments **L+M** and **L+G**. Similarly, a group of 5 cows each from treatments **L+M** and **L+G** were allocated to treatments **L** & **L+G** and **L** & **L+M** respectively (Table 3.1).

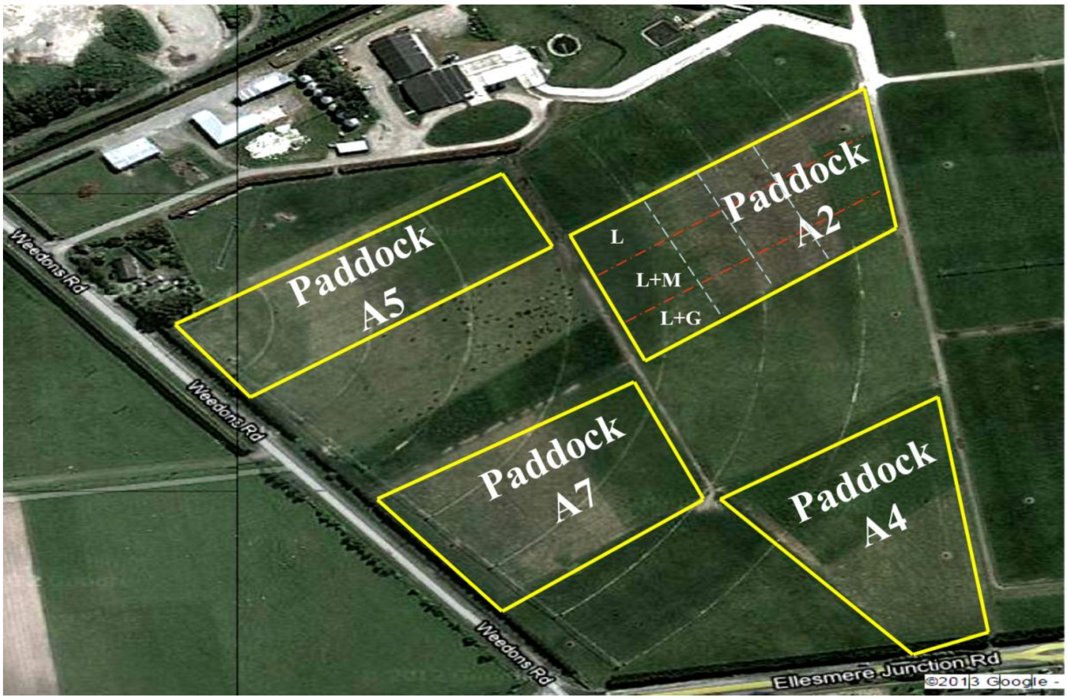


Figure 3.1: Aerial view of Lincoln University Research Dairy Farm (LURDF) map showing experimental area of 4 lucerne paddocks (A2, A4, A5 and A7) and the allocation of lucerne to cows in the three dietary groups (**L**, **L+M** and **L+G**) per paddock.

3.2.1 Maize and grass silage treatments

Ten cows in treatment **L+M** were adapted to maize silage by being offered a starting allowance of 0.6 kg DM/cow/day on day 0 which was increased after every two consecutive days until the target allowance of 3.0 kg DM/cow/day was reached, i.e. from 0.6 to 1.2, 1.8, 2.4 and finally to 3.0 kg DM/cow/day. This was done to prevent incidences of acidosis. Grass silage was offered at the target allowance of 3.0 kg DM/cow/day from day 0. Daily wet weight for both silage treatments was determined by percentage dry matter (%DM) and the required kg DM using the formula:

$$kg\ feed\ wet = kg\ DM\ required\ x\ 100 \div \%\ DM$$

Where; Kg = kilograms
Kg DM = kilograms of dry matter
% DM = percentage of DM in the silage (was determined daily)

Figure 3.2: The dairy cows being fed grass silage (black collar – treatment **L+G**) and maize silage (blue collar – treatment **L+M**) using feeding bins at the LURDF cow-shed.

3.2.2 Grazing management

Uniform management was applied to all cows in all the four paddocks. On the first 7 days, cows in allocated groups, were adapted to lucerne by increasing the allowance of lucerne (offered in morning) by 1 kg DM/cow/day (starting from 10 kg DM/cow/day) with access to water treated with Bloatenz® once daily, (50 ml per 500 kg/liveweight) and Rumensin® Capsule, intra-ruminal bolus to prevent bloat. On day 7, cows were allocated to their full allowance and fenced into daily breaks (including back-fencing) so that cows on all treatments were allocated a daily allowance of 15 kg DM/cow/day above a plot grazing mass of 1500 kg DM/ha in each paddock. Electric fencing was used within paddocks to allocate a fresh break-line of lucerne after each milking. After the afternoon milking, cows back-grazed lucerne that remained from the morning allocation. To optimise on grazing rotation, lucerne pasture was grazed as close as possible to its optimum growth stage in all paddocks, which was about 5 cm of re-growth from the crown in autumn (Basigalup & Ustarroz, 2007).

3.2.3 Measurements

Data collection commenced when the treatments were offered, and continued for a period of 30 days. Measurements were taken on:

i. Dry matter content and quality of lucerne and silages.

ii. Animal parameters included; DMI, grazing behaviour, milk production and composition, N content in urine and faeces, substitution rate and marginal milk response.

3.2.3.1 Feed DM and quality

3.2.3.1.1 Feed samples

Approximately 300 grams of lucerne snip samples were harvested at ground level pre- and post-grazing daily throughout the trial period from 7.30 – 8.00 a.m. (before morning grazing) and immediately transported to be sorted and to estimate the dry matter content and nutritive value. To determine DM content of the lucerne, 100 grams of fresh pre- and post-grazing samples were weighed within 30 minutes after cutting, oven dried at 65°C for 48 hours and reweighed to ascertain dry matter. A sub-sample of approximately 100 grams from pre- and post-grazing herbage was sorted to leaf, stem, grass, clover, weed and dead material for analysis of plant morphology and to determine diet selection. Sorted samples were oven dried at 65°C for 48 hours and botanical composition on a DM basis determined. A second sub-sample of approximately 100 grams was taken from each of the lucerne samples then frozen at -20°C. Approximately 200 grams of maize and grass silage samples were collected every second day, weighed and frozen at -20°C. The second lucerne sub-samples and silage samples were later freeze-dried and ground by a ZM^{200}Retsch grinder (RETSCH, Germany) to 1 mm (Figure B. 5.1), for proximate analysis using wet chemistry procedures.

3.2.3.1.2 Lab analysis of feed samples

Analyses were performed at Lincoln University Analytical Services (Lincoln University, Christchurch, New Zealand). Samples were analysed in duplicate.

NDF was determined according to Van Soest methods of fibre analysis (Van Soest, Robertson, & Lewis, 1991). Neutral detergent fibre (NDF) content of lucerne and grass silage was determined gravimetrically using the method of Van Soest et al

(1991), For maize silage, aNDF was determined by standard alpha-amylase-treated NDF (aNDF) method described by ANKOM (2010), using ANKOM$^{200/220}$ Fiber Analyzer (ANKOM Technology, New York, USA).

ADF was also determined gravimetrically, by extraction with 0.5 M sulphuric acid and cetyltrimethyl-ammonium bromide (CTBA) solution according to procedures described by AOAC (1990).

Metabolisable Energy (ME) was determined from ADF using established regression equation as shown below:

i. Lucerne: ME (MJME/kgDM) = 13.90 - 0.0164[ADF] (Givens, 1989)
ii. Maize silage: ME (MJME/kgDM) = 13.38 - 0.0113[ADF] (Givens, 1990)
iii. Grass silage: ME (MJME/kgDM) = 15.0 - 0.0140[ADF] (Givens, Everington, & Adamson, 1989)

Crude protein was determined by combustion of sample under Oxygen supply and high temperatures using Variomax CN Analyser; Elementar. Ether extracts (EE) were measured by soxhlet extraction in hexane using a BUCHI Soxhlet Extraction Unit E-816HE. Residual dry matter (r-DM) was determined gravimetrically by drying 1g of the sample in a fan forced oven at 100 +/- 5^{0}C for 48 hours or to a constant weight. Ash was determined by subsequent combustion of 1g sample in a muffle furnace (Model LAB 3A KW 5000 W, Max T: 1000 ^{0}C), at 550^{0}C for 4 hours (Figure B. 5.2). Water soluble carbohydrates (WSC) were then calculated using the formula; WSC % = 100% – [CP% + NDF% + EE% + Ash%]

3.2.3.2 Cow measurements and calculations

3.2.3.2.1 Lucerne herbage intake

Apparent DM intake was determined from herbage mass disappearance. Lucerne mass was assessed pre- and post-grazing using a sward height stick. Height was calibrated per lucerne treatment (**L**, **L+M** & **L+G**) to determine DM yield by sampling 30 quadrats each 0.1 m^{2} of pre-and-post grazing herbage to ground level throughout the measurement period. Daily apparent intakes were then estimated using the following equation:

DMI (kg DM/cow/d) = (Pre-Post grazing residual) x Area grazed) ÷ No. of animals

3.2.3.2.2 Diet selection

The concentration of nutrient (N_{sel}; g/kg DM) selected by cows was calculated from previously described equation by Wales, Doyle, and Dellow (1998) as:

Where: M_{pre} and M_{post} are the mass of the pre- and post-grazed herbage (kg DM/ha), and N_{pre} and N_{post} are the concentration of the nutrient (g/kg DM) in the pre- and post-grazed herbage.

Where: M_{pre} and M_{post} are the mass of the pre- and post-grazed herbage (kg DM/ha), and N_{pre} and N_{post} are the concentration of the nutrient (g/kg DM) in the pre- and post-grazed herbage.

The selection differential was calculated as:

$$\text{Selection differential} = N_{sel}/N_{pre}$$

3.2.3.2.3 Lucerne herbage utilisation

Herbage utilization per hectare was defined as herbage intake per hectare expressed as a proportion of pre-grazing herbage mass as:

$$\%\text{Utilization per Ha} = [(\text{Pre} - \text{Post-grazing residual}) \div \text{Pre-grazing mass}] \times 100$$

Herbage utilization per cow was determined as follows:

$$\%\text{Utilization per cow} = [\text{Intake per cow} \div \text{Herbage allowance (kg DM/cow/d)}] \times 100$$

3.2.3.2.4 Grazing behavior

Visual observation of 5 randomly selected cows from each of the three dietary treatments was done simultaneously from 08.24 a.m. to 12.24 p.m. Biting rate (bites/min) was recorded for the 4 hour grazing bout after forage allocation in the morning. Observers counted the number of bites of each cow during one continuous minute at 0, 30, 60, 120, 180 and 240 min after the start of the grazing period. The following activities were also recorded: grazing, walking, ruminating, lying, drinking water and idling/standing (Figure 3.3).

Figure 3.3: Grazing behaviour observation for the dairy cows grazing lucerne (offered at 15 kg DM/cow/day). Activities observed were bites per minute, and time (in minutes) spent grazing, walking, ruminating, lying, drinking water and idling/standing.

3.2.3.2.5 Body condition score & Liveweight gain

On two consecutive days at the beginning (day 1 and 2) and at the end (day 14 and 15) of each period, the body condition of the cows was scored by trained independent observers using the ten-point body condition score (BCs) scale (1 = thin; 10 = obese) (Wildman *et al.*, 1982). Liveweight was recorded daily after the morning (a.m.) and afternoon (p.m.) milking using a walk-over automatic scale.

3.2.3.2.6 Milk production and composition

Milk yield was recorded daily using an automated system (DeLaval Alpro Herd Management System, DeLaval, Tumba, Sweden). Two milk subsamples were collected for every cow at a.m. and p.m. milking on day 0 (baseline), 5, 10 and 15 days so as to determine milk composition and milk urea nitrogen concentration (Figure 3.4).

Figure 3.4: Milk subsamples being collected using milk flasks (with blue cover-lids) during a.m. and p.m. milking. Milk yield was also recorded (light green numerals) by the automated system (DeLaval Alpro Herd Management System, DeLaval, Tumba, Sweden)

Sub-samples which were used to determine milk urea N were centrifuged at 4,000 × g for 10 min at room temperature and refrigerated for 10 min to solidify fat on the top then removed. The skimmed milk was pipetted into a clean micro-centrifuge tube, chilled and transported to the laboratory for immediate analysis. Milk urea nitrogen was analysed commercially (Gribbles Veterinary, Christchurch, New Zealand) on an automated Modular P analyser (Roche Hitachi, Basel, Switzerland) by an enzymatic assay as previously described (Talke & Schubert, 1965). Milk composition was analysed by Livestock Improvement Corporation Ltd laboratory (Christchurch, New Zealand) to determine milk fat, protein and lactose by Milkoscan^{TM} (Foss Electric, Denmark) (Figure 3.5). Milk N output was calculated by dividing the milk protein content (%) by 6.38 to give N (%). This was then multiplied by the milk yield (kg/d) to give the total N output in milk.

Figure 3.5: Milk samples (yellow = p.m. milking and pink = a.m. milking) packed for analysis of milk composition at Livestock Improvement Corporation Ltd laboratory (Christchurch, New Zealand) to determine milk fat, protein and lactose by Milkoscan^{TM} (Foss Electric, Denmark).

3.2.3.2.7 Feed conversion efficiency

Feed conversion efficiency was determined by;-

i. FCE = MS yield (kg/cow/day) ÷ DM intake (kg DM/cow/day); for Milk solids
ii. FCE = Milk yield (kg/cow/day) ÷ DM intake (kg DM/cow/day); for Milk production

3.2.3.2.8 Nitrogen excretion in urine and faeces

Immediately after the morning and afternoon milking on day 0, 5 and 15, cows were herded into the veterinary yards for further sample collection. Urine samples were taken mid-stream after manual stimulation of the vulva. Samples were then acidified below a pH of 4.0 using concentrated sulphuric acid to prevent volatilization, and then frozen at -20°C until analysis. Faeces samples were collected by rectal stimulation or as the animal defecated and frozen at -20°C until analysis.

Urine and faecal samples were analysed at Lincoln University Analytical Services (Lincoln University, Christchurch, New Zealand). Urine samples were analysed for % N, creatinine, urea concentration, purine derivatives and ammonia. Urinary ammonia concentration was analysed by Enzymatic UV Method using Randox Ammonia Kit and urea by Kinetic UV assay using Roche Urea/BUN Kit, both on Roche Cobas Mira plus CC Analyzer. Creatinine concentration of urine was determined by the Jaffé method described by Bartels and Böhmer (1971), using

Cobas Mira Plus Analyzer (Roche Hitachi, Basel, Switzerland). Sub-samples of urine collected on day 15 were analysed for concentration of purine derivatives using HPLC (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) as previously described by Czauderna and Kowalczyk (2000), with modifications by George *et al.* (2006).

Faecal samples were collected by rectal stimulation or as the animal defecated and frozen at -20°C until analysis. The samples were then thawed and subsampled, with one subsample being weighed and oven-dried at 65°C for 48 hours and reweighed to ascertain dry matter. The other sample was freeze dried, ground to 1 mm, and analysed for faecal N% using N-analyser (Vario MAX CN, Elementar Analysensysteme, Hanau, Germany).

3.2.3.2.9 Estimation of microbial N flow

The equations used in calculating the estimated microbial N supply outlined below have been described previously . To estimate the microbial N supply based on the urinary excretion of purine derivatives (**PD**), the PD index was calculated based on total PD [allantoin (mmol/L) + uric acid (mmol/L)] as:

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

The excretion of creatinine (mmol/kg of $BW^{0.75}$) was extrapolated by using the estimated daily urinary volume (L) calculated from the equation by Pacheco, Lowe, Burke, and Cosgrove (2009). The estimated urinary creatinine excretion (0.9 mmol/kg of $BW^{0.75}$) was included in the following equation to estimate the daily excretion of PD (mmol/kg of $BW^{0.75}$):

$$\text{daily excretion of PD (dPD; mmol/kg } BW^{0.75}) = \text{PD index} \times 0.9$$

From this, the amount of purines absorbed daily was estimated:

$$\text{daily absorbed purines (daP)} = [\text{dPD (mmol/kg of } BW^{0.75}) - 0.385 \times BW^{0.75}] + 0.85;$$

Microbial N (g of N/d) supply was determined using the following equation:

$$\text{microbial N (g of N/d)} = (\text{daP} \times 70)/(0.116 \times 0.83 \times 1{,}000)$$

3.2.3.2.10 Substitution rate and milk response to supplementation

Substitution rate was calculated as the reduction in kg lucerne DM per kg of supplement DMI:-

$$= \frac{\text{Mean of treatment group (kgDM/day) - Mean of control group (kgDM/day)}}{\text{Kg DM silage}}$$

Average milk response was then calculated from the extra milk solids produced by total silage intake supplement:-

$$= \frac{\text{Mean of treatment group (kgMS/day) - Mean of control group (kgMS/day)}}{\text{Kg DM silage}}$$

3.2.4 Statistical Analysis

Data were captured and processed with Microsoft Excel 2010/13. Before creating different datasets for analysis, data were checked for anomalies such as duplicate records, incomplete records as well as creating specific animal performance and feed quality variables in separate Microsoft Excel spread-sheets. Statistical analysis was done using GenStat (Release 15 and 16, VSN International Ltd) statistical software. For both animal and feed variables, mean differences between treatment effects was declared significant at probability <0.05 and period × treatment interactions declared at $P < 0.01$. Data analysis was based on the mean of 10 cows in each treatment per each experimental period (1^{st} and 2^{nd} period). The three treatments included lucerne only (L), lucerne + maize silage (L+M) and lucerne + grass silage (L+G). Feed quality data were analysed on chemical composition, botanical composition and DM yield, while cow performance data were analysed on:-

- DMI, MEI, N-intake, subR, utilisation and FCE
- Milk yield, composition (MS, milk protein/fat, milk-N and MUN) and MMR.
- Urine-N, NUE, urine urea, urine NH_3, N% in faeces and urine
- Purine derivatives (allantoin, uric acid) PD index and microbial and creatinine
- Body condition score & live-weight
- Grazing behaviour (height consumed, bites/min, time for grazing, ruminating, idling, lying, walking and drinking water).

The data which were collected at successive days in the 1st and 2nd period on milk yield, composition (MS, milk protein/fat, milk-N and MUN), MMR, urine-N, NUE, urine urea, urine NH_3, creatinine, N% in faeces and urine, and live-weight were analysed by repeated measures ANOVA, where the treatment structure was the 3 dietary treatments × the two periods with no blocking. The ANOVA was adjusted for covariate, in which day 0 was used as the covariate. The level of interaction was set at maximum possible. The ANOVA tables, means, F-probabilities (at $P < 0.05$ and 0.01), level of interaction, standard errors of differences of means (SEM) and the least significance difference (LSD) at 5% were obtained. The LSD was used to separate within row treatment means.

Data on DMI, MEI, N-intake, subR, utilisation, FCE, BCs, diet selection differentials, Purine derivatives (allantoin, uric acid) PD index, microbial-N, and grazing behaviour (height consumed, bites/min, time for grazing, ruminating, idling, lying, walking and drinking water) were analysed by general ANOVA using the two-way ANOVA (no blocking) design in which factors were the 3 dietary treatments and the two periods. ANOVA tables, means, F-probabilities (at $P < 0.05$ and 0.01), level of interaction, standard errors of differences of means (SEM) and the least significance difference (LSD) at 5% were also obtained, and the LSD was used to separate within row treatment means.

Feed quality data on chemical composition, botanical composition and DM yield were analysed by general ANOVA using the general treatment structure (no blocking) design in which the treatment structure for lucerne DM yield was the 3 dietary treatments and the two periods, while for chemical composition and botanical composition the treatment structure was dietary treatments, periods and pre – and post grazing lucerne. ANOVA tables, means, F-probabilities (at $P < 0.05$ and 0.01), level of interaction, standard errors of differences of means (SEM) and the least significance difference (LSD) at 5% were obtained.

Linear polynomial regression was done on the curve obtained for grazing height disappearance, with height (cm) as the response variate and time (seconds) as the explanatory variate. Quadratic polynomial model was used to obtain the F-probabilities ($P < 0.05$), correlations, percentage variance and standard error of observations.

Chapter 4.
RESULTS

4.1 Feed characteristics

4.1.1 Chemical composition

The chemical composition of lucerne herbage during the 1st and 2nd period is shown in Table 4.1. In the 2nd period, lucerne had a reduced stem regrowth compared to the 1st period, which was indicated by a reduction in the overall DM and ADF content in the pre-graze lucerne by 43 and 53 g/kg DM respectively ($P = 0.002$). However, after the regrowth there was a significant increase in the concentration of N and CP ($P = 0.001$) in the 2nd period compared to the 1st period. The change in the amount of ME, ash, OM, WSC, Fat and DMD was not statistically significant ($P > 0.01$). The composition of pre-grazing lucerne across the three dietary treatment allocations did not differ ($P > 0.05$). A comparison of the chemical composition between pre- and post-grazing lucerne herbage shown in Table 4.2, indicated that pre-grazing sward had higher concentration of N, CP, WSC, ME, DMD and fat than the post-grazing lucerne ($P < 0.01$). The amount of ADF and NDF was higher in the post-grazing herbage than the pre-grazing sward ($P < 0.001$). This implied that lucerne parts (mainly leaf) with higher concentrations of N, CP, ME, WSC, fat and DMD were selected more by the grazing dairy cows compared to parts with higher NDF and ADF content (mainly the stem). The composition of post-grazing lucerne herbage did not differ with treatment which indicated the grazing uniformity across the three diets. The DM content was higher ($P < 0.001$) in grass silage (494.1 ± 7.4 g/kg DM) than in pre-grazing lucerne and maize silage (154.2 and 332.1 ± 7.4 g/kg DM respectively). The pre-grazing lucerne (both stem and leaf) had higher contents of CP and N ($P < 0.001$), and lower OM, ether extracts and NDF ($P < 0.001$), compared to maize and grass silage (Table 4.2). The amount of ME was also lower in the pre-grazing lucerne (8.9 ± 0.1 MJ ME/kg DM) compared to the silage supplements. And between the two supplements, grass silage had the highest content of ME compared to maize silage (11.1 vs. 10.2 ± 0.1 MJ ME/kg DM; $P < 0.001$). The concentration ADF in the pre-graze lucerne, maize and grass-silage did not differ significantly ($P > 0.05$).

Table 4:1: Chemical composition (g/kg DM) and ME (MJ ME/kg DM) of the pre-graze lucerne plant (stem and leaf) during the 1st and 2nd period of the autumn experiment.

Component	**Period**		**SEM**	**LSD**	**Main effects**		**Interaction effect**
	1st	2nd			Period	Treatment	Period × treatment
Ash (g/kg DM)	104.4	103.8	±3.5	12.2	NS	NS	NS
OM (g/kg DM)	895.6	896.2	±3.5	12.2	NS	NS	NS
Fat (g/kg DM)	22.4	28.7	±1.9	6.5	NS	NS	NS
DM (g/kg DM)	174.1	131.9	±8.1	24.4	**0.002**	NS	NS
ADF (g/kg DM)	324.9	287.6	±9.6	29.6	**0.002**	NS	NS
NDF (g/kg DM)	391.5	363.8	±11.6	35.8	NS	NS	NS
N (g/kg DM)	34.8	40.3	±0.8	3.8	**0.001**	NS	NS
CP (g/kg DM)	217.3	251.7	±5.5	17.1	**0.001**	NS	NS
WSC (g/kg DM)	264.3	251.9	±6.1	20.9	NS	NS	NS
DMD (g/kg DM)	650.3	645.6	±12.7	44.2	NS	NS	NS
ME (MJ ME/kg DM)	8.6	9.2	±0.2	0.7	NS	NS	NS

DM: Dry matter, OM: Organic matter, EE: Ether Extracts, ADF: Acid detergent fibre, NDF: Neutral detergent fibre, N: Nitrogen, CP: Crude protein, WSC: Water soluble carbohydrates, DMD: Dry Matter Digestibility, ME: Metabolisable energy.

NS = not significant ($P > 0.01$), LSD = Least significant difference (at 5.0%), SEM = Standard errors of means.

Table 4:2: Comparison of the chemical composition (g/kg DM) and ME (MJ ME/kg DM) between pre- and post-grazing lucerne herbage grazed by dairy cows in period 1 and 2 of autumn.

	Pre/post-grazing lucerne				**Main effects**	
Component	Pre-grazing	Post-grazing	**SEM**	**LSD**	Pre/post-graze	Treatment
Ash (g/kg DM)	104.1	103.8	±3.3	10.3	NS	NS
OM (g/kg DM)	895.9	896.2	±3.3	10.3	NS	NS
Fat (g/kg DM)	25.5	15.8	±1.4	4.5	**<0.001**	NS
DM (g/kg DM)	154.2	199.8	±6.6	19.3	**<0.001**	NS
ADF (g/kg DM)	306.2	463.5	±9.5	29.5	**0.002**	NS
NDF (g/kg DM)	377.6	573.8	±11.6	35.7	**<0.001**	NS
N (g/kg DM)	37.5	22.5	±0.8	2.7	**<0.001**	NS
CP (g/kg DM)	234.5	140.9	±5.5	17.1	**<0.001**	NS
WSC (g/kg DM)	258.2	165.7	±6.8	21.2	**<0.001**	NS
DMD (g/kg DM)	647.9	508.8	±7.1	21.8	**<0.001**	NS
ME (MJ ME/kg DM)	8.9	6.3	±0.2	0.5	**<0.001**	NS

DM: Dry matter, OM: Organic matter, EE: Ether Extracts, ADF: Acid detergent fibre, NDF: Neutral detergent fibre, N: Nitrogen, CP: Crude protein, WSC: Water soluble carbohydrates, DMD: Dry Matter Digestibility and ME: Metabolisable energy.
NS = not significant (P > 0.01), LSD = Least significant difference (at 5.0%).

Table 4:3: The chemical composition (g/kg DM) and (ME (MJ ME/kg DM**)** of pre-grazing lucerne, maize and grass silage offered to mid-lactating dairy cows in autumn. Lucerne represents the weighted average of leaf and stem for period 1 and 2.

	Diets					
Component	Pre-grazing lucerne	Maize silage	Grass silage	**SEM**	**LSD**	***P*-value**
Ash (g/kg DM)	104.1^{a}	41.2^{c}	80.5^{b}	±1.7	6.4	**<0.001**
OM (g/kg DM)	895.9^{c}	958.8^{a}	919.5^{b}	±1.7	6.4	**<0.001**
Fat (g/kg DM)	25.6^{c}	27.0^{b}	39.2^{a}	±1.5	5.9	**<0.001**
DM (g/kg DM)	154.2^{c}	332.1^{b}	494.1^{a}	±7.4	32.2	**<0.001**
ADF (g/kg DM)	306.3	280.7	277.9	±8.8	33.9	NS
NDF (g/kg DM)	377.6^{b}	$492.4^{*\ a}$	498.0^{a}	±10.2	39.5	**<0.001**
N (g/kg DM)	37.5^{a}	12.5^{c}	22.2^{b}	±1.0	3.9	**<0.001**
CP (g/kg DM)	234.5^{a}	77.9^{c}	138.5^{b}	±6.4	24.6	**<0.001**
WSC(g/kg DM)	258.2^{b}	361.4^{a}	243.8^{c}	±8.2	31.5	**<0.001**
DMD (g/kg DM)	647.9^{b}	598.8^{c}	753.7^{a}	±6.8	26.3	**<0.001**
ME (MJ ME/kg DM)	8.9^{c}	10.2^{b}	11.1^{a}	±0.1	0.5	**<0.001**

DM: Dry matter, OM: Organic matter, EE: Ether Extracts, ADF: Acid detergent fibre, NDF: Neutral detergent fibre, N: Nitrogen, CP: Crude protein, WSC: Water soluble carbohydrates, DMD: Dry Matter Digestibility, ME: Metabolisable energy, $_{*}$ =aNDF,
NS = not significant ($P > 0.05$)
P-value = significance level for comparison of means ($\alpha < 0.05$)
^{a,b,c} means within a row with different superscripts differ ($P < 0.05$).

4.1.2 Plant morphology and DM yield

4.1.2.1 Lucerne botanical characteristics

The sward comprised almost entirely of lucerne (88%) with the remainder (12%) comprising of clover (1.8%), grass (0.4%), weeds (1.8%) and dead matter (8%). Botanical composition on DM-basis of pre- and post-grazing lucerne sward during period 1 and 2 is presented in Table 4.4. Pre-graze lucerne had an almost equal DM content of leaf (47.13%) and stem (40.62%), resulting to a leaf:stem ratio of 1.13. After grazing, the post-grazing lucerne had a higher content of stem DM (68.03%) than leaf DM (11.78%), which resulted to a lower leaf:stem ratio of 0.18. This also implied that cows consumed more lucerne leaf compared to stem. The content of dead material was high ($P < 0.001$) in the post-grazing lucerne due to the high amount of stem that was left behind after grazing. The increased content of dead material in the 2nd period was attributed to the greater amount of stem left in the post-grazing residual of the 1st period when stem heights were high. And since topping-up of the lucerne pasture was not done after grazing in the 1st period, this could have also contributed to the increased dead material in the pre-graze lucerne of the 2nd period. The slow growth of lucerne in the 2nd period compared to the 1st period reduced the content of stem DM from 58.16% to 48.73% ($P < 0.001$). Consequently, the pre-grazing ratio of leaf:stem increased from 0.99 to 1.30 ($P = 0.003$) in the 2nd period. However, the slow regrowth did not reduce the content of leaf DM ($P > 0.05$). The amount of clover, grass and weed did not differ ($P > 0.01$) between the pre- and post-graze sward and between the two periods. Similarly, the botanical composition of the lucerne herbage (pre- and post-grazing) did not differ ($P > 0.05$) across the three treatment allocations in the paddocks from where the snip samples were obtained.

Table 4:4: Percentage botanical composition and leaf-stem ratio on DM-basis of pre- and post - grazing lucerne sward offered at an allowance of 15 kg DM cow^{-1}day^{-1} to mid-lactation dairy cows in the 1st and 2nd period of autumn.

Variable	**Pre/post – grazing lucerne**		**Period**		**SEM**	**LSD**	**Main effects**		
	Pre-graze	Post-graze	1st period	2nd period			Pre/post-graze	Period	Treatment
Stem fraction (%)	40.62	68.03	58.16	48.73	± 1.81	5.26	**<0.001**	**<0.001**	NS
Leaf fraction (%)	47.13	11.78	29.60	31.07	± 1.19	3.48	**<0.001**	NS	NS
Dead material (%)	8.19	17.74	8.26	17.65	± 1.32	3.85	**<0.001**	**<0.001**	NS
Clover (%)	2.07	1.56	2.21	1.41	± 0.53	1.54	NS	NS	NS
Grass (%)	0.39	0.27	0.37	0.30	± 0.17	0.51	NS	NS	NS
Weed (%)	1.61	0.63	1.41	0.85	± 0.37	1.08	NS	NS	NS
Leaf-stem ratio	1.13	0.18	0.99	1.30	± 0.04	0.14	**< 0.001**	**0.003**	NS

NS = not significant (*P* > 0.01)

4.1.2.2 Dry matter (DM) yield

Lucerne had an average DM yield of 3,107.3 ± 104.6 kg DM ha^{-1} in autumn (Table 4.5). The DM yield was assessed pre- and post-graze, and calibration equation was used to estimate the pre-grazing mass. The regression equation obtained for the pre-grazing lucerne in autumn was described as shown below (Figure 4.1)

$y = 57.047x + 81.734$; $R^2 = 0.78$ ($P < 0.001$)

Where; y = estimated pre-grazing mass (kg DM/ha), x = sward height (cm),

R^2 = regression coefficient (78%). The standard error of observation was ± 42.

Table 4.5 indicates that low heights and DM yield were obtained in the 2nd period compared to the 1st period (31.4 vs. 58.9 ± 1.8 cm and 2,598.5 vs. 3,446.5 ± 104.6 kg DM/ha; $P < 0.001$). This reduction was attributed to the slow regrowth of lucerne during the 2nd period. The estimated grazed mass per hectare was higher ($P < 0.001$) in the 1st period when the pre-grazing lucerne had a higher mass compared to the 2nd period when lucerne herbage mass was low. DM yield of lucerne did not differ between treatments ($P > 0.05$), and the allocation of treatments in the paddocks had no effect ($P > 0.05$) on the regrowth of lucerne in the second period.

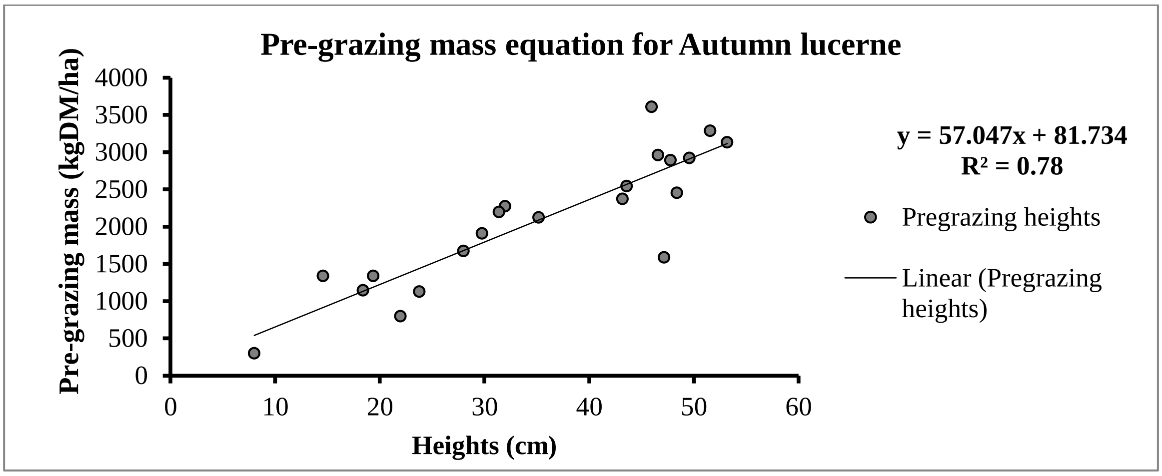


Figure 4.1: Regression equation that was used to estimate pre-grazing mass (kg DM/ha) of lucerne in autumn. The equation was obtained from calibration of pre-grazing sward heights (●) by sampling 30 quadrats each 0.1 m^2 of pre-grazing herbage to ground level.

Table 4:5: Average pre- and post - graze heights (cm), DM yield (kg DM/ha) and grazed (kg DM/ha) for lucerne pasture that was offered to mid-lactation dairy cows during the 1st and 2nd period of the experiment conducted in autumn.

Variable[1]	**Period**		**Mean**	**SEM**	**LSD**	**Main effects**		**Interaction**
	1st period	2nd period				Period	Treatment	T×P
Pre-grazing height (cm)	58.9	31.4	47.9	±1.8	4.8	**<0.001**	NS	NS
Post-grazing height (cm)	25.6	15.9	21.7	±0.8	2.6	**<0.001**	NS	NS
Pre-grazing mass (kg DM/ha)	3,446.5	2,598.5	3,107.3	±104.6	273.2	**<0.001**	NS	NS
Post-grazing mass (kg DM/ha)	1,785.2	1,582.5	1,704.1	±51.0	133.2	**0.004**	NS	NS
Grazed mass (kg DM/ha)	1,661.3	1,016.1	1,403.2	±94.5	246.8	**<0.001**	NS	NS

[1] variable – includes accumulated daily measurements of three diet treatments (lucerne only, lucerne + maize silage and lucerne + grass silage)

4.2 Animal performance

4.2.1 Dry Matter (DM) intake and nutrient selection

The measurements on DMI (kg DM/cow/day), MEI **(**MJ ME/kg DM/cow/day), percentage utilisation and SubR (kg DMI of lucerne/kg DMI silage are presented in Table 4.6. Estimated average DMIs for lucerne per cow were slightly higher ($P = 0.043$) for cows in the control group offered lucerne only (14.14 ± 0.57 kg DM/cow/day) compared to those supplemented with maize and grass silage (12.77 and 12.91 ± 0.574 DM/cow/day respectively. The intake of silage increased the total DMI for the supplemented cows compared the lucerne only cows ($P = 0.021$). The total DMI for cows offered maize and grass silage averaged at 15.49 and 15.58 ± 0.41 kg DM/cow/day. Although cows offered maize silage had a higher intake of the supplement than the grass silage fed cows (2.82 vs. 2.67 ± 0.02 kg DM/cow/day; $P < 0.001$), the total DMI did not differ between the supplemented cows. The ME intake was in the range of 124.12, 112.77 and 114.08 ± 3.52 MJ ME/kg DM/cow/day for cows offered lucerne only, lucerne + maize silage and lucerne + grass silage respectively and did not differ across the diets (P > 0.05). However, the total MEI from lucerne and supplements was significantly higher ($P < 0.001$) for cows receiving maize and grass silage (140.52 and 144.83 ± 3.51 MJ ME/kg DM/cow/day respectively) compared to the lucerne only cows (124.13 ± 3.52 MJ ME/kg DM/cow/day). And despite the higher MEI intake from grass silage ($P < 0.001$), the total MEI did not differ in the supplemented cows. The period effect was significant ($P < 0.001$) on MEI from lucerne and the subsequent total MEI, in which cows had higher intakes in the 2nd period across the three diets. As shown in Table 4.6, the selected lucerne ME did not differ across the diets ($P > 0.05$); however, more ME was selected in the 2nd period compared to the 1st period ($P < 0.001$). The total MEI from the selected lucerne and silage supplements was significantly high ($P < 0.001$) for the supplemented cows compared to the lucerne only cows, with intakes being in the range of 171.29, 186.61 and 194.68 ± 3.96 MJ ME/kg DM/cow/day for cows receiving lucerne only, lucerne + maize silage and lucerne + grass silage respectively. The reduction in lucerne intake by up to1.36 kg DM/cow/day resulted in subR of 0.44 ± 0.04 kg DMI of lucerne/kg DMI silage, but which did not differ between cows offered either maize or grass silage. However, in the 1st period, subR was significantly higher ($P < 0.001$) compared to the 2nd period. The average utilisation of lucerne per hectare was generally low, ranging from 41.5% to 47.2% ± 1.9%, compared to the high utilisation per cow which ranged from 85.2% to

94.3% ± 3.8%. The dietary treatments had no effect on both utilisations. Period effect was significant on the utilisation per hectare ($P < 0.001$), whereby the utilisation was lower in the 2nd period compared to the 1st period.

Table 4.7 provides a summary on the concentration of nutrient in lucerne that was offered, and the estimated selection differentials and nutrient intake of dairy cows grazing the lucerne. Nutrient selection did not differ across the three diets ($P > 0.05$), however, there was a significant period effect ($P < 0.01$) on the selection of CP, N, ME, fat, WSC, DMD, ADF and NDF. Cows selected lucerne with higher concentration of CP, N, ME, fat, WSC and DMD in the 2nd period, while NDF and ADF were consumed more in the 1st period. This effect of period on selection of lucerne nutrients was attributed to the reduction in stem growth in the 2nd period consequently, reducing the concentration of ADF and NDF. On average, cows in all the three diet groups consumed lucerne with 1.55 ± 0.19 times more crude protein (CP) and nitrogen (N) than the herbage on offer. Selection differentials were also high for fat (1.52 ± 0.18), water soluble carbohydrates (1.49 ± 0.17) and ME (1.28 ± 0.10). In contrast to CP and N, cows consumed herbage with less ADF and NDF than that in the offered herbage, and which was implicated to the selection of more leaf than stem. The selection differentials for ADF and NDF were 0.30 ± 0.24 and 0.29 ± 0.25 respectively. The amount of ash and organic matter consumed was almost equal to that in offered lucerne. Apparent dry matter digestibility for the dairy cows was 1.29 ± 0.10 times more than the estimated *in vitro* DM digestibility.

Table 4:6: The measurements on DMI (kg DM/cow/day), MEI (MJ ME/kg DM/cow/day), percentage utilisation and SubR (kg DMI of lucerne/kg DMI silage for mid-lactation dairy cows grazing lucerne in autumn supplemented with either maize or grass silage.

Measurement	**1st Period**			**2nd Period**			**Average**			**SEM**	**LSD**	**Main effects**	
	L	L+M	L+G	L	L+M	L+G	L	L+M	L+G			Trt	Period
Lucerne DMI (kg DM/cow/d)	13.96	12.32	12.27	14.40	13.45	13.87	14.14^{a}	12.77^{b}	12.91^{b}	±0.57	1.16	**0.043**	NS
Supplement DMI (kg DM/cow/d)	-	2.72	2.77	-	2.90	2.77	-	2.72	2.67	±0.02	0.06	**<0.001**	NS
Total DMI (kg DM/cow/d)	13.96	15.04	15.04	14.40	16.35	16.64	14.14^{b}	15.49^{a}	15.58^{a}	±0.41	1.16	**0.021**	NS
Lucerne MEI (MJ ME/kg DM)	119.72	105.61	105.21	130.74	123.51	127.41	124.12	112.77	114.08	±3.52	10.06	NS	**<0.001**
Supplement MEI (MJ ME/kg DM)	-	27.76	30.74	-	34.65	23.85	-	27.76	30.75	±0.67	1.27	**<0.001**	NS
Total MEI (MJ ME/kg DM)	119.72	133.37	135.95	130.74	158.16	151.26	124.12^{b}	140.53^{a}	144.83^{a}	±3.52	10.06	**<0.001**	**<0.001**
Selected lucerne MEI (MJ ME/kg DM)	162.13	151.48	151.42	185.04	163.02	182.72	171.29	158.85	163.93	±3.96	11.34	NS	**<0.001**
Total selected MEI (MJ ME/kg DM)	162.13	179.23	182.16	185.04	197.67	206.57	171.29^{b}	186.61^{a}	194.68^{a}	±3.96	11.34	**<0.001**	**<0.001**
Utilisation per Hectare (%)	52.5	45.0	44.6	39.2	40.8	36.9	47.2	43.3	41.5	±1.9	5.4	NS	**<0.001**
Utilisation per cow (%)	93.1	82.2	81.8	96.0	89.7	92.5	94.3^{a}	85.2^{b}	86.1^{b}	±3.8	4.9	**0.043**	0.033
SubR (kg DMI of lucerne/kg DMI silage)	-	0.61	0.61	-	0.33	0.21	-	0.41	0.47	±0.04	0.11	NS	**<0.001**

L = lucerne only, L+M = lucerne + maize silage, L+G = lucerne + grass silage, Trt = treatment, SEM = Standard errors of treatment means,
LSD = Least significant difference (at 5.0%)
^{a,b}means within a row with different superscripts differ ($P < 0.05$).

Table 4:7: Nutrient concentration of lucerne herbage offered, estimated selection differentials and nutrient intake of grazing dairy cows.

Component/nutrient	Total nutrient selected[1] (g/kg DM)	Selection differential[2]	Nutrient intake (g/day)[3]	Nutrient selected per period		SEM	Main effects		
				Period I	Period II		Trt	Period	T×P
Nitrogen	58.02 ± 2.36	1.55 ± 0.19	765.11 ± 10.21	55.82	61.33	±1.97	NS	**	NS
Crude protein	362.57 ± 14.72	1.55 ± 0.19	4780.92 ± 63.75	348.81	383.19	±12.27	NS	**	NS
Fat	39.01 ± 1.54	1.52 ± 0.18	514.48 ± 6.79	37.57	41.17	±1.28	NS	**	NS
WSC	384.76 ± 14.55	1.49 ± 0.17	5076.05 ± 65.91	371.17	405.15	±12.13	NS	**	NS
ME (MJ ME/kg DM)	12.46 ± 0.31	1.28 ± 0.10	1646.91 ± 19.66	12.16	12.90	±0.26	NS	**	NS
In vitro DMD	84.46 ± 21.90	1.29 ± 0.10	NA[4]	843.01	888.17	±18.25	NS	**	NS
Ash	104.51 ± 0.01	1.00 ± 0.00	13877.69 ± 17.77	104.47	104.58	±0.01	NS	NS	NS
Organic matter	895.49 ± 0.05	1.00 ± 0.00	11891.30 ± 152.93	895.53	895.42	±0.04	NS	NS	NS
ADF	91. 21 ± 24. 73	0.30 ± 0.24	1267.64 ± 99.06	114.31	56.56	±20.61	NS	**	NS
NDF	109.15 ± 30.86	0.29 ± 0.25	1519.85 ± 123.21	137.98	65.90	±25.72	NS	**	NS

Significance level: NS = $P > 0.05$; * = $P < 0.01$; ** = $P < 0.01$; *** = $P < 0.001$
Trt = treatment effect, T×P = treatment × period interaction effect

[1] Nutrient selected by cow determined as [(mass of pre-graze herbage × concentration of the nutrient in pre-graze herbage) – (mass of post-graze herbage × concentration of the nutrient in post-graze herbage)] ÷ (mass of pre-graze herbage – mass of post-graze herbage) × 10

[2] Selection differential = Nutrient selected ÷ concentration of the nutrient in pre-graze herbage

[3] Values are mean ± SEM

[4] NA means not applicable

4.2.2 Milk yield and composition

The average measurements on milk yield and composition, MMR, FCE, LWT and body condition of the dairy cows during the 1st and 2nd period are presented in Table 4.8 and 4.9. Effect of the dietary treatments on daily milk yield, milk-solids, milk fat and protein was non- significant ($P > 0.05$) except for MUN which was higher in dairy cows offered lucerne only (15.85 ± 0.37 mmol/L; $P = 0.044$) compared to cows supplemented with either grass or maize silage (15.30 and 14.94 ± 0.37 mmol/L respectively). The interaction between period and treatment was significant ($P < 0.01$) on milk yield, milk-solids, milk fat and milk protein in which higher yields were obtained in the 1st period compared to the 2nd period. Time also had a significant effect ($P \leq 0.001$) on milk yield, milk-solids, milk-fat and milk-protein in that, there was general initial decline in production of milk-solids, milk-fat and milk-protein from day 5 to 10 after diets were offered; and from day 10 to 15, the yield increased (Figure 4.2). Although milk yield did not show any logical trend, the average yields at day 15 were higher than at day 0 for the three diets. There was a significant interaction between time and treatment ($P \leq 0.002$) for milk yield and milk-protein in that towards the end of the trial, milk-protein and milk yield were on a decline for cows offered lucerne only compared to the supplemented cows. The interaction between time and period was significant ($P < 0.001$) for milk yield, milk-solids, milk-fat and MUN, whereby, the increases in milk yield with time were greater in the 1st period than in the 2nd period; whereas, milk-solids, milk-fat and MUN gradually increased in the 1st period and decreased in the 2nd period for day 1 to 15. Covariate effect was significant ($P < 0.001$) on milk yield, MUN and LWT i.e., the initial status of cows at the beginning of the experiment, whereby cows which had high initial milk production, MUN and LWT at the onset of the trial ended-up having higher average values compared to cows that started at low level of milk yield, MUN and LWT. The average marginal milk-solids response was 0.02 ± 0.01 kg MS per kg DM silage intake, which did not differ with the type of silage ($P > 0.05$).

4.2.1 Feed conversion efficiency, Body condition and Liveweight

Summary on the calculated FCE (kg MS per kg DMI lucerne/cow/day for the dairy cows is shown in Table 4.8 and 4.9. Statistical analysis showed no significance difference in the efficiency of converting feed to milk-solids ($P > 0.05$) between cows offered lucerne only, lucerne + maize silage and lucerne + grass silage. However, there was a significant period effect ($P < 0.001$) on the efficiency. Cows had higher FCE in the 1st period compared to 2nd period (0.11 vs. 0.08 ± 0.01 kg MS per kg DMI lucerne/cow/day). The effect of the dietary treatments on liveweight gain and body condition was positive but non-significant ($P > 0.05$). Over the two experiment periods (total of 30 days), cows gained body condition from 4.03 to 4.18, and liveweight from 476.88 to 480.20 kg. There was no significant interaction ($P > 0.01$) between the dietary treatment and period on liveweight and body condition.

Table 4:8: Milk yield and composition, marginal milk-solids response (kg MS/DMI silage), feed conversion efficiency (kg MS/kg DMI), LWT (kg) and body condition during period 1 and 2 for mid-lactation Friesian-Jersey dairy cows grazing lucerne in autumn and supplemented with grass or maize silage.

	1st Period			**2nd Period**			**Average**				**Main effects**		**Interaction**
Parameter	L	L+M	L+G	L	L+M	L+G	Period 1	Period 2	**SEM**	**LSD**	Trt	Period	P×Trt
Milk yield (kg/day)	15.74	14.85	15.72	13.71	14.24	14.58	15.44	14.18	±0.26	0.73	NS	**0.001**	NS
Milk-solids (kg/day)	1.56	1.59	1.58	1.28	1.28	1.32	1.58	1.28	±0.03	0.04	NS	**<0.001**	NS
Milk fat (kg/day)	0.90	0.91	0.92	0.70	0.70	0.73	0.91	0.71	±0.02	0.06	NS	**<0.001**	NS
Milk protein (kg/day)	0.66	0.68	0.66	0.58	0.58	0.59	0.67	0.57	±0.01	0.02	NS	**<0.001**	NS
MUN (mmol/L)	16.33	15.08	15.42	15.38	14.79	14.79	15.61	15.12	±0.39	0.77	**0.044**	NS	NS
MMR(kgMS/DMI silage)	-	0.02	0.01	-	0.01	0.03	0.01	0.02	±0.01	0.05	NS	NS	NS
FCE (kg MS/kg DMI)	0.12	0.10	0.10	0.09	0.08	0.08	0.11	0.08	±0.01	0.01	NS	**<0.001**	NS
LWT (kg)	478.60	477.50	478.90	483.60	482.30	480.20	478.30	482.00	±2.49	7.06	NS	NS	NS
Body condition	3.98	4.00	4.13	4.10	4.25	4.20	4.03	4.18	±0.15	0.21	NS	NS	NS

L = lucerne only, L+M = lucerne + maize silage, L+G = lucerne + grass silage, Trt = treatment, P×Trt = period × treatment interaction.
SEM = Standard errors of treatment means
LSD = Least significant difference (at 5.0%)
P-value for comparison of treatment means (α <0.05)
^{a,b,}means within a row with different superscripts differ ($P < 0.05$).

Table 4:9: The average milk yield and composition, marginal milk-solids response (kg MS/DMI silage), feed conversion efficiency (kg MS/kg DMI), LWT (kg) and body condition in the 1st and 2nd period for mid-lactation Friesian-Jersey dairy cows grazing lucerne in autumn and supplemented with grass or maize silage

Parameter	Average performance per diet			SEM	LSD	Main effects			Interaction effects		
	L	L+M	L+G			Trt	Time	Covariate	T×Trt	T×P	T×Trt×P
Milk yield (kg/day)	14.72	14.54	15.15	±0.31	0.88	NS	**<0.001**	**<0.001**	**<0.001**	**<0.001**	NS
Milk-solids (kg/day)	1.41	1.43	1.45	±0.04	0.10	NS	**<0.001**	NS	NS	**<0.001**	NS
Milk fat (kg/day)	0.79	0.80	0.83	±0.02	0.07	NS	**0.001**	NS	NS	**<0.001**	NS
Milk protein (kg/day)	0.62	0.63	0.62	± 0.02	0.05	NS	**<0.001**	NS	**0.002**	NS	NS
MUN (mmol/L)	15.85^{a}	14.94^{b}	15.30^{a}	±0.37	0.74	**0.044**	NS	**<0.001**	NS	**<0.001**	NS
MMR(kg MS/DMI silage)	-	0.01	0.02	±0.01	0.05	NS	-	-	-	-	-
FCE (kg MS/kg DMI)	0.10	0.09	0.09	±0.01	0.01	NS	-	-	-	-	-
LWT (kg)	481.10	479.90	479.60	±3.04	8.62	NS	NS	**<0.001**	NS	NS	NS
Body condition	4.04	4.13	4.16	±0.09	0.26	NS	NS	NS	NS	NS	NS

Trt = treatment, T×Trt = time × treatment interaction, T×P = time × period interaction, T×Trt×P = time × treatment × period interaction

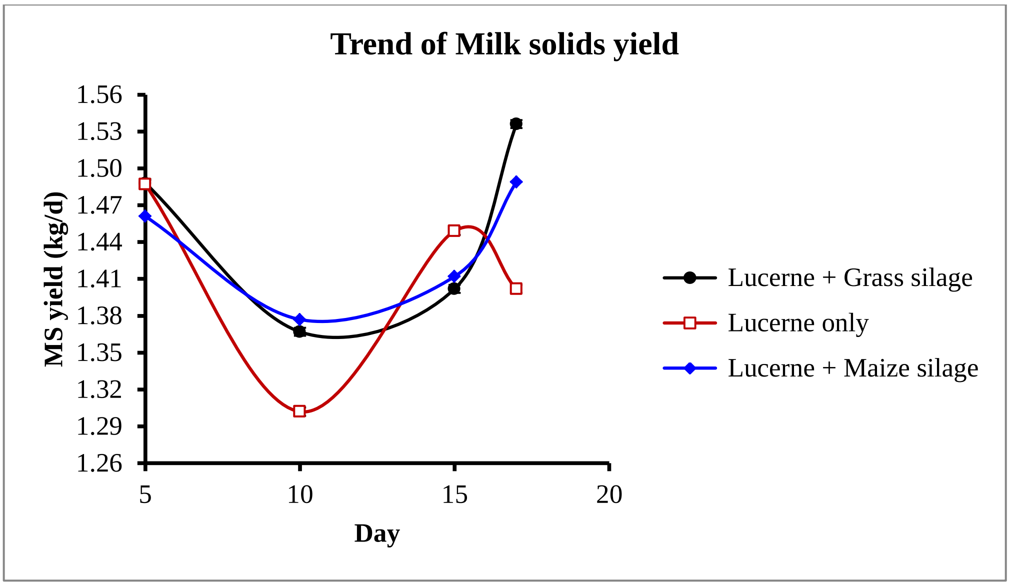


Figure 4.2: The trend of milk solids production from day 5 to 17 of the autumn experiment for dairy cows grazing lucerne and supplemented with either grass silage or maize silage. The effect of time was significant at $P < 0.001$.

4.2.2 Grazing behavior

Summarized grazing behaviour data are presented in Figure 4.3, Table 4.10 and 4.11. Figure 4.3, illustrates the treatment effect on pasture disappearance. The height of lucerne gradually decreased with each bite taken after every 15 minutes of measurement. And within the first grazing session of 4 hours after being offered a new allocation (08:24 a.m. to 12:24 p.m.), cows in the control group (lucerne only) had consumed 24 cm or 51% of their total DMI, which was higher ($P < 0.001$) compared to that of cows receiving maize silage (24 cm or 49%) and grass silage (19 cm or 39%). The standard error of the height measurements was ± 5.64 and the percentage variance accounted for was 73.6%. Generally, from the summarized grazing behaviour parameters in Table 4.11, a significant effect ($P < 0.05$), was observed of treatment on the measured grazing parameters i.e., number of bites per minute, time spent grazing, idling, lying, walking, drinking water and pasture consumed with the exception of time spent ruminating. During the 2nd period when the growth of lucerne was reduced, cows had higher number of bites per minute ($P < 0.001$), and spent more time grazing ($P < 0.001$) and foraging ($P = 0.005$; Table 4.10) compared to the 1st period when lucerne heights were higher. The effect of period on pasture consumed and time spent idling, ruminating, lying and drinking water was non-significant ($P > 0.01$).

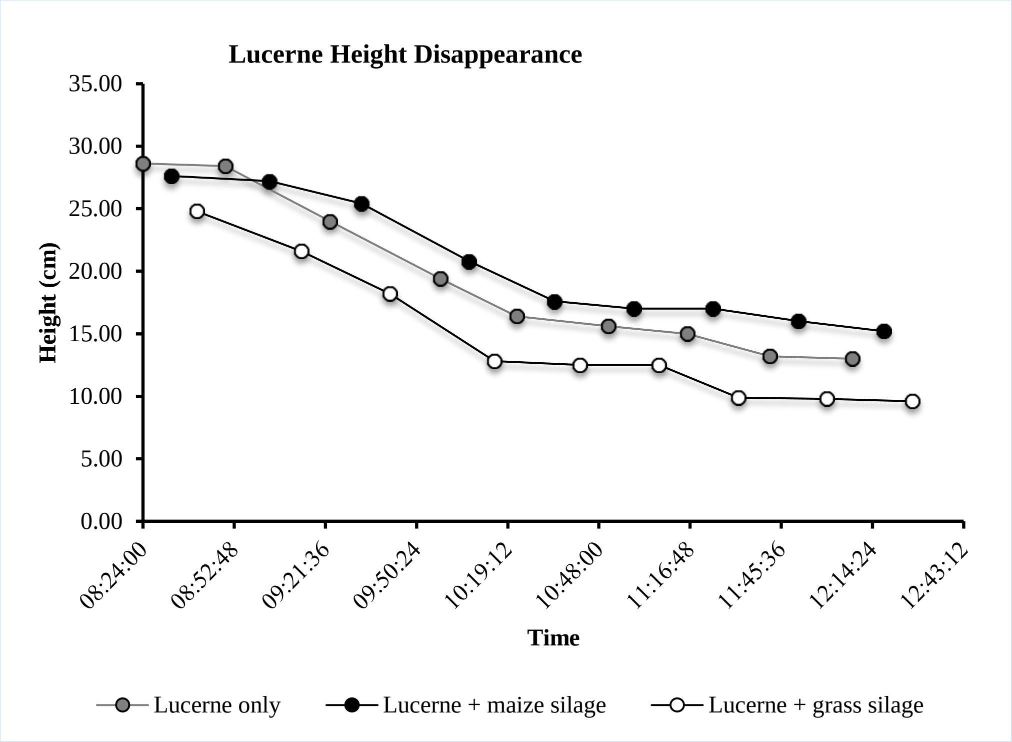


Figure 4.3: The trend of lucerne pasture height disappearance for mid-lactation cows fed lucerne only (●), lucerne + maize silage (●) and lucerne + grass silage (○) in autumn. Standard error of observations = ± 5.64 and the variance accounted for in taking the heights = 73.6%.

Table 4:10: Summary of grazing behaviour parameters within the first morning grazing session of four hours for dairy cows fed lucerne during the 1st and 2nd period of the autumn experiment.

	Period				
Measurement	1st Period	2nd Period	**SEM**	**LSD**	***P*-value**
No. of bites/min	30	41	±2	4	**<0.001**
Height consumed (cm)	45	28	±7	20	NS
Grazing time (minutes)	165	182	±5	15	**<0.001**
Idling (minutes)	18	9	±6	17	NS
Ruminating (minutes)	12	10	±1	2	NS
Lying (minutes)	14	8	±2	7	NS
Walking/foraging (minutes)	14	24	±3	10	**0.005**
Drinking water (minutes)	12	7	±2	6	NS

NS = not significant ($P > 0.01$)

Table 4:11: Summary of grazing behaviour parameters within the first morning grazing session of four hours measured on herbage consumed, number of bites/minute, time spent (in minutes) on grazing, idling, ruminating, walking and drinking water for mid-lactation cows offered lucerne in autumn and receiving grass or maize silage.

Measurement	**Diet**			**SEM**	**LSD**	***P*-value**
	Lucerne only	Lucerne + maize silage	Lucerne + grass silage			
Herbage consumed (%)	51%^{a}	49%^{a}	39%^{b}	±1	2.1	**<0.001**
No. of bites/minute	39^{a}	34^{b}	39^{a}	±2	4.1	**0.013**
Grazing time (minutes)	120^{a}	113^{b}	114^{b}	±1	0.6	**<0.001**
Idling (minutes)	7^{b}	9^{a}	11^{a}	±2	3.6	**0.004**
Ruminating (minutes)	7	8	7	±1	1.4	NS
Lying (minutes)	0^{b}	22^{a}	0^{b}	±1	1.7	**<0.001**
Walking (minutes)	15^{a}	10^{b}	13^{a}	±1	2.2	**0.019**
Drinking water (minutes)	7^{a}	7^{a}	5^{b}	±1	1.6	**0.023**

^{a,b,}means within a row with different superscripts differ ($P < 0.05$).
NS = not significant ($P > 0.05$)

4.2.3 Nitrogen concentration in urine and faeces

Summary on nitrogen intake and output is presented in Tables 4.12 and 4.13. The amount of pre-grazing lucerne herbage N intake differed (*P* = 0.043) across the dietary treatments from 479.43 to 530.77 ± 15.25 g N/cow/day. The intake supplement N also differed between cows offered maize and grass silage (48.346 and 52.96 ± 1.16 g N/cow/day; *P* = 0.030), and which increased the total N intake for the supplemented cows. However, the total N intake did not differ between the three diets (*P* > 0.05). The estimated selection differentials showed higher consumptions of N (mainly from leaf) than that in the whole herbage on offer (stem + leaf). Consequently, higher selected lucerne N intakes were estimated, with cows receiving lucerne only having higher intakes (820.52 ± 23.58 g N/cow/d; *P* = 0.043) compared to cows fed lucerne + maize and silage and lucerne + grass silage (741.14 and 749.32 ± 23.58 g N/cow/d). Supplementary N intake also increased the total selected N intake for supplemented cows, but this did not significantly differ from that of lucerne only cows. The period effect and the interaction between period and treatment were significant (*P* > 0.05) on the supplement N intake, whereby, cows had higher N intakes in the 2nd period. Generally, the effect of dietary treatments on most of the measured nitrogen parameters pertaining to N excretion in milk, urine and faeces was non-significant (*P* > 0.05), except for urinary and faecal N output. The diets had no effect on the concentration of urea in milk and urine, the urinary concentrations of creatinine, purine derivatives (PD) i.e. allantoin and uric acid, calculated PD index and the total microbial N supply (*P* > 0.05). The calculated nitrogen use efficiency (NUE) and milk N content did not differ across the three dietary treatments (*P* > 0.05). As indicated in Table 4.12, the estimated nitrogen excretion through urine (N g/day) and faeces (%N) varied across the three dietary treatments, with cows offered lucerne only having greater urinary-N excretion (434.62 ± 18.99 g/day; *P* = 0.010) compared to those supplemented with maize and grass silage (365.28 and 352.52 ± 18.99 g/day respectively; Figure 4.4). The type of silage had no effect on urinary-N output. The calculated % reduction in urinary N excretion for the supplemented cows compared to the control group was 19% and 16% for cows offered grass silage and maize silage respectively. The faecal N output was lower in cows grazing lucerne only (*P* = 0.002) compared to those receiving supplement. Cows consuming grass silage excreted higher faecal N content of 2.98 ± 0.07% N (DM) than cows fed maize silage (2.83 ± 0.07%N (DM); Figure 4.5). The increase in faecal N output in the supplemented cows in comparison to control group averaged at 8.71% and 4.03% for cows fed grass and maize silage respectively. There was a significant period effect ($P < 0.01$) on the content of purine derivatives i.e. allantoin, and uric acid, total purine derivatives (PD), total microbial N and PD index. Cows had higher values for PD derivatives, microbial N supply and PD index in the 1st period compared to those in the 2nd period. Covariate effect was significant ($P \leq 0.002$) on milk-N, NUE, urine and milk urea. Time had a significant effect ($P \leq 0.009$) on the concentration on milk-N, milk urea, urine NH_3, urine %N and the NUE, in that the concentration of urine NH_3, milk-N, urine %N and NUE increased from day 1 to day 15, while milk urea declined gradually. Interaction between time and period was significant ($P \leq 0.010$) for milk-N, milk urea, urine urea and NUE, whereby milk-N and NUE gradually increased from day 5 to 15 in the 1st period and declined in the 2nd period, whereas, milk urea decreased in the 1st period and increased with time in period 2. The increase in the concentration of urine urea was higher in period 1 than 2. The interaction between time, period and treatment was significant ($P < 0.001$) on milk urea.

Table 4:12: Nitrogen intake (g N/cow/day) and nitrogen parameters pertaining to N excretion in milk, urine and faeces of cows grazing 15 kg DM/cow/d of lucerne and supplemented with 3 kg DM/cow/d of grass silage or maize silage in autumn.

Measurement	**Feed**			**SEM**	**LSD**	**Main effects**		**Interaction**
	L	L+M	L+G			Treatment	Period	Trt ×Period
Lucerne herbage N intake (g/cow/d)[1]	530.78^{a}	479.43^{b}	484.72^{b}	±15.25	43.63	**0.043**	NS	NS
Supplement N intake (g/cow/d)	-	48.35	52.96	±1.16	4.14	**0.030**	**<0.001**	**<0.001**
Total N intake (g/cow/d)[2]	530.78	527.78	537.68	±15.25	43.63	NS	NS	NS
Selected lucerne N intake (g/cow/d)[3]	820.52^{a}	741.14^{b}	749.32^{b}	±21.51	67.45	**0.043**	NS	NS
Total selected N intake (g/cow/d)[4]	820.52	789.49	802.28	±23.58	67.45	NS	NS	NS
Milk Urea (mmol/L)	7.94	7.59	7.64	±0.12	0.35	NS	NS	NS
Milk N (g/day)[5]	92.87	94.42	93.53	±3.27	6.56	NS	NS	NS
NUE (%)[6]	17.80	17.89	17.40	±0.16	1.52	NS	NS	NS
Urine NH_3 (mmol/L)	1.08	1.05	1.24	±0.15	0.29	NS	NS	NS
Urine Urea (mmol/L)	174.24	161.87	167.74	±7.82	22.17	NS	NS	NS
Urine N%	0.49^{a}	0.41^{c}	0.47^{b}	± 0.01	0.03	**<0.001**	NS	NS
Urine N (g/day)	434.63^{a}	365.28^{b}	352.52^{b}	±18.99	53.85	**0.010**	NS	NS
Fecal %N (DM)	2.72^{c}	2.83^{b}	2.98^{a}	±0.07	0.13	**0.002**	NS	NS
Creatinine (mmol/L)	1.16	1.16	1.29	±0.08	0.15	NS	NS	NS
Allantoin (mmol/L)	10.53	8.95	9.80	±0.74	2.11	NS	**<0.001**	NS
Uric acid (mmol/L)	1.15	0.99	1.00	±0.08	0.22	NS	**<0.001**	NS
Total PD (mmol/L)[7]	11.68	9.94	10.80	± 0.79	2.23	NS	**<0.001**	NS
PD index[8]	1087.22	900.71	864.92	± 91.20	258.57	NS	**0.004**	NS
Microbial supply[9]	683.33	561.27	537.96	±59.56	258.57	NS	**0.004**	NS

^{a,b,c}means within a row with different superscripts differ ($P < 0.05$), Trt = treatment

[1] Lucerne herbage N intake = [Crude Protein × DMI] ÷ 6.25

[2] Total N intake = Lucerne herbage N intake + Supplement N intake

[3] Selected lucerne N intake = Nitrogen selected determined as [(mass of pre-graze herbage × concentration of the nutrient in pre-graze herbage) – (mass of post-graze herbage × concentration of the nutrient in post-graze herbage)] ÷ (mass of pre-graze herbage – mass of post-graze herbage) × 10

[4] Total selected N intake = Selected lucerne N intake + Supplement N intake

[5] Milk N = (milk protein content (%) ÷ 6.38) × milk yield (kg/d)

[6] NUE = milk N ÷ Total N intake (g/cow/d) × 100

[7] Total PD = allantoin + uric acid.

[8] PD index = [(total PD)/creatinine] × $BW^{0.75}$ (kg).

[9] Determined assuming daily purine derivative excretion (dPD; mmol/kg of BW0.75) = PD index × 0.9; daily absorbed purines (daP) = [dPD (mmol/kg of $BW^{0.75}$) − 0.385 × $BW^{0.75}$ + 0.85; and microbial N (g of N/d) = (daP × 70)/(0.116 × 0.83 × 1,000).

Table 4:13: Nitrogen intake and concentration in milk, urine and faeces during the 1st and 2nd period in autumn of the experiment.

	Period				**Main effects**		**Interaction effects**			
Measurement	1st Period	2nd Period	**SEM**	**LSD**	Period	Time	T×Trt	T×P	T×Trt×P	Covariate
Lucerne herbage N intake (g/cow/d)	482.44	496.60	11.37	36.36	NS	-	-	-	-	-
Supplement N intake (g/cow/d)	44.63	59.27	1.31	3.87	**<0.001**	-	-	-	-	-
Total N intake (g/cow/d)	527.07	555.87	11.37	36.36	NS	-	-	-	-	-
Selected lucerne N intake (g/cow/d)	734.94	781.61	17.58	56.21	NS	-	-	-	-	-
Total selected N intake (g/cow/d)	779.57	840.88	21.53	56.21	NS	-	-	-	-	-
Milk Urea (mmol/L)	7.80	7.65	0.13	0.36	NS	**<0.001**	NS	**<0.001**	**<0.001**	**<0.001**
Milk N (g/day)	95.81	91.40	3.27	4.06	NS	**<0.001**	NS	**<0.001**	NS	**0.002**
NUE (%)	17.88	17.79	0.37	1.07	NS	**<0.001**	NS	**<0.001**	NS	**0.002**
Urine NH_3 (mmol/L)	1.06	1.18	0.12	0.24	NS	**<0.001**	NS	NS	NS	NS
Urine Urea (mmol/L)	168.20	167.61	7.79	12.03	NS	NS	NS	**0.010**	NS	**<0.001**
Urine %N	0.44	0.48	0.01	0.03	NS	**0.009**	NS	NS	NS	NS
Urine N (g/day)	364.68	403.60	15.51	43.97	NS	NS	NS	NS	NS	NS
Faecal %N (DM)	2.87	2.81	0.06	0.11	NS	NS	NS	NS	NS	NS
Creatinine (mmol/L)	1.22	1.19	0.06	0.13	NS	**0.005**	NS	NS	NS	NS
Allantoin (mmol/L)	11.31	8.20	0.61	1.72	**<0.001**	**-**	**-**	**-**	**-**	**-**
Uric acid (mmol/L)	1.21	0.89	0.06	0.18	**<0.001**	**-**	**-**	**-**	**-**	**-**
Total PD (mmol/L)	12.52	9.09	0.64	1.82	**<0.001**	**-**	**-**	**-**	**-**	**-**
PD index	1107.12	794.78	74.46	211.12	**0.004**	**-**	**-**	**-**	**-**	**-**
Microbial N (g N/d)	696.62	491.74	68.78	137.89	**0.004**	**-**	**-**	**-**	**-**	**-**

Trt = treatment, T×Trt = time × treatment interaction, T×P = time × period interaction, T×Trt×P = time × treatment × period interaction

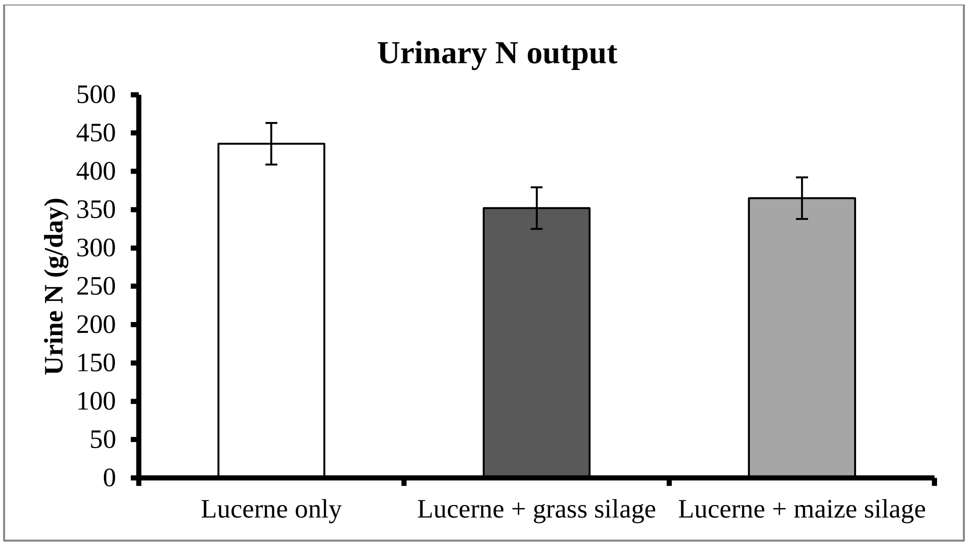


Figure 4.4: Nitrogen output in urine (g/day) for cows fed lucerne pasture only (□), lucerne + grass silage (■) and lucerne + maize silage (■) in autumn. The symbol ┬ on each bar is the SEM = ±18.99, LSD = 53.85 and *P* = 0.010.

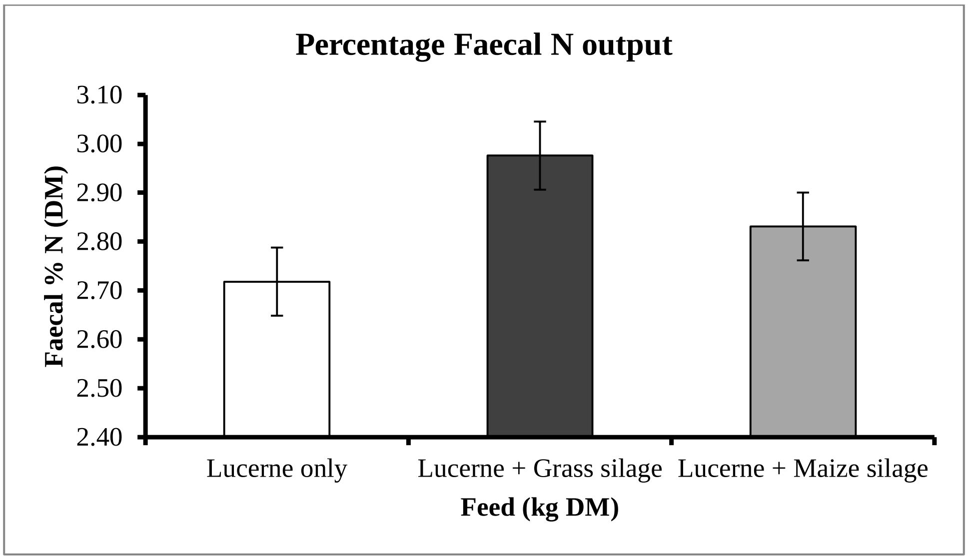


Figure 4.5: Faecal N output (%N/DM) for cows grazing lucerne only (□), lucerne + grass silage (■) and lucerne + maize silage (■) offered in autumn. The SEM (┬) is ±0.07, LSD = 0.14 and *P* = 0.002.

Chapter 5.

DISCUSSION

5.1 General overview

The main aim of this research was to investigate the effect of supplementary feed on DMI, substitution rate, milk-solids and nitrogen excretion of lucerne fed dairy cows (Chapter 1). To achieve this aim, an experiment was conducted to quantify dry matter intake (DMI), urinary and faecal-N output, milk production and composition, SubR and marginal milk response (MMR) for mid-lactation dairy cows grazing lucerne and supplemented with maize or grass silage in autumn (Chapter 3). The experiment comprised of three treatments consisting of lucerne, maize and grass silage randomly allocated to 30 mid-lactation Friesian-Jersey dairy cows in an incomplete randomised crossover design in two periods each of 15 days with an adaptation phase and washout period of 7 days each. This chapter discusses results (Chapter 4) in relation to previous work (Chapter 2) and provides conclusions and recommendations.

5.2 Feed characteristics

5.2.1 Chemical components of lucerne sward and supplement

Pre-grazing lucerne herbage constituted of 52% leaf and 48% stem on DM-basis. The chemical components of the pre-grazing lucerne in g/kg DM consisted of DM = 154.2 ± 7.4, CP = 234.5 ± 6.6, N = 37.5 ± 1.0, NDF = 377.6 ± 11.6, ADF = 306.2 ± 9.5, WSC = 258.2 ± 6.8, fat = 25.6 ± 1.4, ash = 104.1 ± 3.3, OM = 895.9 ± 3.3, DMD = 647.9 ± 7.1 and ME of 8.9 MJ ME/kg DM. These quality parameters fall within the range of typical feed values summarized for lucerne forage by Woodward *et al.* (2010), Castillo (1999), Hill laboratories (2013), Burke (2004) and Chatepa (2012). However, the ME value of lucerne herbage in the present study was slightly lower compared to that reported by Brown and Moot (2004) and Woodward *et al.* (2010) of 11.6 and 10.6 MJ ME/kg DM respectively for lucerne produced under irrigation conditions in autumn.

The concentration of N was higher ($P < 0.001$) in lucerne forage (37.5 ± 1.0 g/kg DM) compared to that in maize silage (12.5 ± 1.0 g/kg DM) and grass silage (22.2 ± 1.0 g/kg DM), which coincided with that reported in previous grazing studies investigating the effects of offering a range of pasture or forage and low-N silage supplements on dry matter intake, milk production and, or nitrogen utilisation of grazing dairy cows; in which supplementary feeds had lower N content (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007; Morrison & Patterson, 2007; Tamminga, Bannink, Dijkstra, & Zom, 2007; Woodward *et al.*, 2002a; Woodward, Chaves, Grayling, & Waghorn, 2001).

5.2.2 Growth characteristics of lucerne

Lucerne had reduced regrowth in the 2nd period, towards late autumn (April-May) compared to the 1st period in early autumn (February-March). This resulted in general decrease in stem height and pre-grazing mass ($P < 0.001$; Table 4.5). The decreased stem regrowth was also reflected by the reduction ($P < 0.01$) in the composition of ADF of the lucerne plant (Table 4.1). This reduced growth was also evidenced by the increase in leaf:stem ratio from 0.99 to 1.30 ± 0.04 in the 2nd period ($P = 0.003$; Table 4.4). This growth characteristic of lucerne in autumn has been described previously by Moot *et al.* (2003). The author reported that during autumn, the ratio of shoot to root production decreases. And the decreasing temperatures and photoperiods at this time of the year tend to reduce both growth and development. During autumn, the main allocation of assimilates for the lucerne crop shifts from shoot to root production, replenishing reserves for overwintering and spring regrowth. This reduced regrowth of lucerne in present study caused changes in the chemical composition (mainly ADF, N and CP) of lucerne resulting to significant period effect on most of the measured animal parameters.

5.3 Animal Performance

5.3.1 Nutrient selection

There is little quantitative data on the selection of nutrients by dairy cows grazing irrigated lucerne in autumn when offered supplementary feed. In the current study, the effect of dietary treatments on nutrient selection was non-significant ($P > 0.05$), however, there was a significant period effect ($P < 0.01$) on the selection of CP, N, ME, fat, WSC, DMD, ADF and NDF. Generally, the cows selected lucerne with higher concentrations of CP, N, ME, fat, WSC and DMD in the 2nd period, while NDF and ADF were consumed more in the 1st period. This effect of period on selection of lucerne nutrients was attributed to the reduction in stem growth in the 2nd period. Overall, cows consumed lucerne parts (mainly leaf) with higher ME of 12.5 ± 0.3 MJ/kg DM which was 1.28 ± 0.10 times more than 8.9 MJ ME/kg DM in the herbage on offer. Cows also consumed greater amounts of CP and N of up to 362.6 ± 14.7 and 58.0 ± 2.4 respectively, which was 1.55 ± 0.19 times more than that in the offered herbage. Selection differential was also high for fat (1.52 ± 0.18) and water soluble carbohydrates (1.49 ± 0.17; Table 4.7). In contrast, cows consumed herbage with 0.30 times less ADF and NDF than that on offer. The implication being that, cows selected more leaf than stem, which had higher content of CP, N and ME than NDF and ADF. This also coincided with the low leaf:stem ratio for post-grazing lucerne ($P < 0.001$). Similar findings on higher leaf consumption compared to stem have been reported in previous studies with cattle grazing lucerne (Popp *et al.*, 1999; Schlegel, Wachenheim, Benson, Ames, & Rust, 2000; Smith *et al.*, 2013). Clark, Lambert, Rolston, and Dymock (1982), suggested that livestock tend to select more green leaf than on offer in the sward because it is of higher preference than dead herbage or because it is more accessible in the upper grazed horizon of the canopy. This selection of green leaf may also reflect the lower structural strength and sheer force of green leaf, and hence ease of prehension (Hendricksen & Minson, 1980).

Grazing trials involving grass-pastures in which selection differential was estimated, found lower differential values for *in vitro* DMD, CP and ME compared to those obtained in the current study with cows grazing lucerne forage. The selection differential for the *in vitro* DMD in the present study was higher by up to + 0.17 over that reported for most grass pastures. The selection differentials for *in vitro* DMD were estimated to be 1.12 for cows grazing irrigated perennial ryegrass–white clover swards in spring and autumn (Wales *et al.*, 1998; Wales, Stockdale, Doyle, & Dellow, 1999), for dairy cows grazing rain-fed perennial pastures at different herbage allowances in spring (Moate, Dalley, Roche, & Grainger, 1999) and those of Kellaway, Tassell, Havilah, Sriskandarajah, and Andrews (1993) for irrigated ryegrass–kikuyu swards throughout the year. The selection differential for CP of 1.55 ± 0.19 was higher compared to 1.32 reported by Moate *et al.* (1999), 1.33 obtained by

Wales *et al.* (1999), 1.35 measured by Kellaway *et al.* (1993) and that within the range of 1.24 – 1.48 reported by Wales *et al.* (1998). These high selection differentials for CP in the present study implied that the CP intake exceeded the general recommended requirements of about 16% in mid lactation (Kolver, 2000; NRC, 1989; Waghorn, Burke, & Kolver, 2007). The requirements for CP were exceeded by up to 14.26% CP (DM). The selection differential for ME (1.28 ± 0.10) was also higher compared to that reported by Kellaway *et al.* (1993) of 1.16 for cows grazing ryegrass–kikuyu pastures. The higher selection differentials for DMD and ME for dairy cows in the present study coincided with the implications of selective grazing for forage summarised by Cosgrove and Edwards (2007), which states that the digestibility and ME of the diet selected is generally higher than the whole pasture on offer (Guy, Watkin, & Clark, 1981; L'Huillier, Poppi, & Fraser, 1984).

5.3.2 Herbage intake and Utilisation

5.3.2.1 Dry matter intake and Substitution rate

Usually, supplementary feeds are offered to grazing dairy cows to increase dry matter (DM) and metabolisable energy (ME) intakes; however, offering feed supplements reduces pasture DM intake, a phenomenon known as substitution (Bargo *et al.*, 2003; Sheahan, Kolver, & Roche, 2011; Stockdale, 2000). In the present study, silage supplementation, regardless of silage type, increased ($P < 0.05$) both total dry matter intake (DMI) and metabolisable energy intake - MEI (MJ ME/kg DM) when compared with the control lucerne only treatment (Table 4.6). The intake of the silage supplements increased the total DMI to 15.49 and 15.58 ± 0.41 kg DM/cow/day for cows fed lucerne + maize silage and lucerne + grass silage respectively compared to the cows offered lucerne only (14.14 ± 0.57 kg DM/cow/day). Previous studies under lucerne grazing supplemented with corn grain also reported increased total daily intakes ranging from 16.6, 16.7 and 19.4 kg DM cow^{-1}day^{-1} for cows offered lucerne only, lucerne + 3.5 kg DM of corn cow^{-1}day^{-1} and lucerne + 7.0 kg DM of corn cow^{-1}day^{-1} respectively in autumn of 1991, and 16.8, 17.8, 18.1 and 18.5 for cows offered lucerne only, lucerne + 3.0 kg DM of corn cow^{-1}day^{-1}, lucerne + 6.0 kg DM of corn cow^{-1}day^{-1} and lucerne + 9.0 kg DM of corn cow^{-1}day^{-1} respectively in autumn of 1992 (Castillo, Romero, Quaino, Comerón, & Gaggiotti, 2001a). The silage supplements also increased the total MEI to 140.53 and 144.84 ± 3.52 MJ ME/kg

DM/cow/day for cows offered maize and grass silage respectively compared to 124.13 ± 3.52 MJ ME/kg DM cow^{-1}day^{-1} for cows fed lucerne only. The estimated selection differentials suggested that cows selected lucerne with higher ME content, hence the total MEI was substantially high within the range of 171.29, 186.61 and 194.68 ± 3.96 MJ ME/kg DM cow^{-1}day^{-1} for cows receiving lucerne only, lucerne + maize silage and lucerne + grass silage respectively.

Apart from increasing the total DMI and MEI of supplemented cows, offering the silage supplements reduced lucerne pasture DM intake by up to 1.36 kg DM/cow/day, resulting in lucerne DMI of 12.78 and 12.92 ± 0.57 kg DM/cow/day for cows offered maize and grass silage respectively. Un-supplemented cows consumed more lucerne (14.14 ± 0.57 kg DM/cow/day; $P = 0.043$) than the supplemented cows. Previous studies have reported intakes within similar ranges for un-supplemented cows grazing lucerne. Danelon, Locatelli, Gallardo, and Guaita (2002) reported intakes of 13.11 ± 0.63 and 10.52 ± 0.57 kg DM/cow/day for Argentine Holstein dry cows offered 15 kg DM/cow/d lucerne wilted to 35–40% DM) and fresh lucerne respectively. Smith *et al.* (2013) and Woodward *et al.* (2010) reported higher intakes of 15.2 and 18.0 kg DM/cow/day for dairy cows offered higher allowances of 17 and 20 kg DM/cow/d in early and late lactation respectively. The implication being that at higher allowances (>15 kg DM), cows are likely to have higher DMI. Smith *et al.* (2013), found no significant difference ($P > 0.05$) in DMI between cows offered lucerne and ryegrass.

The reduction in lucerne intake resulted in substitution rate of 0.44 ± 0.04 kg DMI of lucerne/kg DMI silage, which did not differ between cows fed either maize silage or grass silage ($P > 0.05$) This rate of substitution obtained in the present study is lower compared to 0.66 kg DM pasture per kg DM corn reported for Holstein dairy cows grazing lucerne in autumn (Castillo *et al.*, 2001a), and much lower than 0.84 to 1.02 kg of grass DM kg^{-1} of forage supplement DM for dairy cows grazing ryegrass dominant pasture (Bargo *et al.*, 2003). Although, the exact mechanisms for this low subR were not determined, it could be attributed to the low supplement allowance (3 kg DM/cow/d), in addition to the fact that cows were in mid lactation when the high energy intake is partitioned more towards foetal growth and body reserves, less to milk (Alderman, 1983). The low subR did not however translate to higher milk solids response to silage supplement as it would have been expected. Nonetheless, the substitution can be managed for the benefit of the farm, through deliberately sparing lucerne pasture that can be used at a later time (e.g. during winter or early spring) with minimal loss in quality. In this case, supplements are fed when lucerne growth rate is lower than the herd's feed demand (kg DM/ha daily), in order to maintain DM intakes, lactation length and milk production, while maintaining or increasing average pasture cover through reduction in pasture consumption. In the latter situation feeding supplements is a deliberate and managed substitution to replace grazed lucerne pasture, with the aim of maximising the growth of high quality lucerne while maintaining feeding levels. This positive effect of "managed" substitution was shown by Grainger and Mathews (1989), Wales, Williams, and Doyle (2001), and Wills and Holmes (1988), where cows offered a restricted pasture allowance plus supplement consumed less pasture but as much energy as cows offered a higher pasture allowance with no supplement. Similarly, supplemented cows in the present study consumed less lucerne pasture due to substitution, but with MEI that did not differ from that of un-supplemented cows. Thus, in this study, substitution can be utilised to allocate a lesser lucerne allowance of 12 kg DM/cow/d plus 3 kg DM/cow/d of silage instead of the 15 kg DM/cow/d lucerne, consequently, sparing up to 3 kg DM/cow/d lucerne, and which could increase the rotational length from 30 to 35 days. Such increase in the inter-grazing interval would allow adequate regrowth time for lucerne to accumulate adequate pre-graze herbage of high quality before the next grazing; this is particularly significant during seasons of reduced lucerne regrowth in autumn and early spring.

5.3.2.2 Percentage utilisation

The % utilisation of lucerne per hectare across the three diets was low compared to the high utilisation per cow, averaging at 44 ± 2% vs. 88.5 ± 3.8% respectively. Such low utilisation rates per hectare for cows grazing lucerne have been reported previously. Dougherty *et al.* (1988), reported that cows consumed herbage at a greater rate when utilisation per hectare (efficiency of harvesting per hectare) was low at 44%. High rates of utilisation per hectare (>50%) were found to reduce individual cow intake rate and general animal performance (Popp *et al.*, 1997). Cangiano, Castillo, Guerrero, and Putnam (2008), explained that the low % utilisation per hectare and the high utilisation per cow was due to trade-off between complete forage utilization (yield and harvest efficiency) through intensive grazing and the animal´s performance, daily gain, or milk production. With dairy grazing systems, there is a clear trade-off between maintaining high milk production through grazing high-quality lucerne forage and more intensive grazing to maximize harvesting efficiency per hectare (Cangiano *et al.*, 2008). Mostly, high pasture utilisation per cow like that observed in the present study is achieved through less grazing pressure (lax grazing), which usually results in low pasture utilisation per hectare. Intensive grazing tends to maximize forage yield and harvesting efficiency per hectare (grazing plants completely to the ground). However, grazing lucerne when the pasture consists mainly of low-quality stems at the bottom of the canopy forces the cows to consume a diet with significantly lower quality. This decreases total daily intake, affecting body weight gain, milk yield and utilisation per animal (Cangiano *et al.*, 2008).

Thus, cows in the present study, under lax grazing, consumed more of the higher parts of lucerne sward, leaving low-quality stems at the bottom of the canopy, which resulted in the high per cow utilisation (DM-intake) and the low per hectare utilisation. It has been suggested that the low utilisation of lucerne per hectare can be improved by using leader-follower grazing systems (Blaser, 1982; Dougherty *et al.*, 1988), whereby the follower animals have lower nutrient requirements (e.g., dry cows or cow-calf pairs). This prevents wasting the lower quality stem material and allows pasture to be grazed with improved efficiency (complete forage utilisation).

5.3.2.3 Feed conversion efficiency to Milk-solids

Although the total DMI and MEI was high in the supplemented cows, the efficiency of converting the feed to milk-solids did not differ ($P > 0.05$) between cows offered lucerne only and those fed maize or grass silage. Thus, the supplements did not improve the FCE, and which can be partly attributed to the physiological status of the dairy cows during lactation. Changes in FCE during the course of lactation have been well documented (Beever & Doyle, 2007). Typically, FCE declines as lactation progresses (Kirkland & Gordon, 2001; Phyn, Clark, Aspin, & Kolver, 2008) as a result of nutrients being used for processes other than milk-solids production during mid to late lactation. Kirkland and Gordon (2001), found partitioning of MEI to milk energy to significantly decrease with advance in lactation from early to late lactation. Often, after peak lactation (mid-late lactation), milk yield declines and cows enter a state of positive energy balance and commence tissue repletion. In the present experiment, cows were in mid-late lactation when milk yield was on a decline before drying-off in April-May, and which could have resulted in the lack of improvement in the FCE for cows offered maize or grass silage (0.09 ± 0.01 kg MS/kg DMI) compared to cows grazing lucerne only (0.01 ± 0.01 kg MS/kg DMI).

5.3.3 Milk production and composition

5.3.3.1 Marginal milk-solids response

There was no significant effect ($P > 0.05$) observed of the dietary treatments on daily production of milk yield and milk-solids production, with the average yield being in the range of 14.81 ± 0.31 kg milk/day and 1.43 ± 0.04 kg MS/day respectively. The milk-solids yield in the present study are lower compared to 1.61 and 2.23 kg MS/cow/day for dairy cows offered 17 kg DM/cow/day of lucerne in autumn and spring respectively (Smith *et al.*, 2013), suggesting the effect of lucerne allowance and season on milk-solids yield. Generally, the marginal milk-solids response was low, averaging 0.02 ± 0.01 kg MS per kg DM silage intake, which did not differ with the type of silage ($P > 0.05$). This low response in autumn could be attributed to the steady decline in milk yield during mid - late lactation (Alderman, 1983). However, response rates are determined by more factors than just the cow's physiological response to supplements. Penno, Holmes, MacDonald, and Walsh (1998), concluded that the largest increase in milk solids production from supplementation would occur at times of greatest underfeeding (relative energy deficit), irrespective of the season or lactation stage. Implying that cows in the current study were not underfed, and that the consumption of energy from lucerne herbage was adequate to meet the ME requirements for milk-solids yield. Hence, the extra MEI from silage supplements was partitioned more towards replenishing body reserves and for foetal growth (pregnancy). According to the estimates of total ME requirements for dairy cows, which constitute maintenance, milk production, change in liveweight, walking and pregnancy (DairyNZ, 2012; Holmes *et al.*, 2002; Kolver, 2000), milking cows require $\simeq$ 49 to 68 MJ ME/day for maintenance needs related to liveweight (0.6 MJ ME/kg $LW^{0.75}$) that ranges from 350 to 550 kg LWT respectively. Approximately, 65 MJ ME is required to produce 1 kg MS, 39 MJ ME is required for a milking cow to gain 1kg liveweight, whereas, 32 MJ ME is released when 1kg liveweight is lost. Gaining one condition score is equivalent 25 kg liveweight gain in a Jersey cow, and 35kg in a Friesian cow. To walk 1 kilometre on flat land, a cow uses 1 MJ ME, which increases to 5 MJ ME per kilometre on hilly terrain. In early pregnancy, the growing calf requires little extra energy from its mother to grow. However, for the last 4 months of pregnancy (i.e. mid-late lactation), the energy demands become more significant, ranging from 5, 10, 20 and 30 MJ ME/day in the 6th, 7th, 8th and 9th month of pregnancy respectively. Based on these estimates for ME requirements, it implies that cows in the present study had their daily ME requirements largely met at an average MEI of 124.12 MJ ME/kg DM from lucerne only (i.e. the intake for the control group). Thus, the extra MEI from silage supplements was approximately 16.41 and 20.71 MJ ME/kg DM for maize and grass silage respectively. Out of this extra MEI, about 10 MJ ME/day or 0.90 kg DM grass silage and 0.98 kg DM maize silage was partitioned towards foetal growth (since on average the cows were in the 7th month of pregnancy). The remaining extra MEI was partitioned towards replenishing body reserves ($\simeq$ 5 to 9 MJ ME/day) and MS yield (1.3 MJ ME/kg DM to produce the additional 0.02 kg MS). This implies that in mid-lactation, less extra MEI (< 65 MJ ME) from supplementary feeds is partitioned towards MS production resulting in low MMR, and which can be attributed to the steady decline in milk yield from the 6th month of pregnancy.

Under the spring-calving pattern in New Zealand's pasture-based dairy systems, autumn has often been promoted as the most economical season to feed supplements (de Klerk, 2012). This is not because cows are more efficient at converting supplementary feed to milk during mid-late lactation, but rather due to a system response, i.e. as pasture growth slows, extra supplement such as grain or silage can be used to extend the round and protect the residual, effectively growing more grass/lucerne and allowing more pasture to be harvested over a longer period, thereby increasing days in milk and milk production per cow (de Klerk, 2012). Thus, the low efficiency of converting feed to milk-solids and the subsequent low MMR for cows fed silage in the present study, indicate that supplementary feeds can be used in autumn to sustain high DM intakes (White, 1982) and enable substitution of the sown lucerne crop to increase herbage cover on the farm during periods of reduced growth in autumn/winter and early spring.

5.3.4 Liveweight change and Body condition

Feeding maize and grass silage supplements resulted in positive liveweight gain and body condition but which was non-significant ($P > 0.05$). Liveweight was gained at the rate of +0.25 kg/cow/day, and body condition added at the rate of +0.15 units per cow/day. These findings are consistent with those from a review by Broster and Broster (1998), which found little variation among treatment groups in LWT and body condition during mid to late lactation, and attributed this to partitioning of nutrients and energy balance. During mid-late lactation, cows are usually in positive energy balance and partitioning of energy intake is more towards foetal growth and LWT gain and less to milk yield (Alderman, 1983). In the present study however, supplemented cows which had higher total MEI and low MMR, ended up with LWT and BC which did not differ from the un-supplemented cows. It could have been expected that the extra MEI not used for milk-solids yield be partitioned towards gain of body weight and body condition. Holden, Muller, Lykos, and Cassidy (1995), also found lack of overall significant differences in body condition score for Holstein cows grazing grass with maize silage. The authors attributed this to be partly due the short duration of the trial and noted that supplemental silage may have a positive effect on body condition through an entire lactation but may not have an effect over a short period. Similarly, in the present study, the lack of dietary effect on LWT and BC can partly be due to the short duration of the trial (30 days), and partly due to partitioning of more energy intake towards foetal growth in mid-late lactation. The estimates of total ME requirements for dairy cows indicate that 39 MJ ME is required for a dairy cow to gain 1kg liveweight and to gain one condition score is equivalent 25kg liveweight gain in a Jersey cow, and 35kg in a Friesian cow (DairyNZ, 2012; Holmes *et al.*, 2002). In present study, cows partitioned $\simeq$ 10 MJ ME/day towards foetal growth and $\simeq$ 5.11 to 9.41 MJ ME/day from the silage supplements to replenish body reserves. This amount of energy was therefore not adequate for the supplemented cows to gain 1kg liveweight or one condition score compared to the un-supplemented cows over the short (30 days) duration of the experiment. However, over an entire lactation, the accumulated energy could have a positive effect on body condition, LWT and MS yield for the supplemented cows.

Thus, it could be postulated that the benefit of offering maize or grass silage in autumn is when the carryover effects of substituted lucerne pasture and spared cow condition are turned to milksolids after calving in spring. Clark (1993), noted that supplements fed in the spring are likely to benefit only when offered early during a period of severe underfeeding (e.g. in autumn) and if they are stopped before the end of the feed deficit.

5.3.5 Grazing behaviour

The height of lucerne gradually decreased with each bite taken after every 15 minutes of measurement (Figure 4.3).Within the morning grazing session of 4 hours, after being offered a new allocation in the morning, cows in the lucerne only group had consumed 51 ± 1% of their total DMI, higher ($P < 0.001$) than those offered maize silage (49 ± 1%) or grass silage (39 ± 1%). Overall, cows had consumed 46 ± 1% of their total daily intake. This percentage consumption for cows within the 1st grazing session is much lower compared to 66% to 69% reported for ryegrass (Gregorini *et al.*, 2009) and chicory diets (McCoy, Collins, & Dougherty, 1997), and 57% for simple and diverse swards in autumn (Bryant, Miller, & Edwards, 2012). The structure of lucerne canopy could have constrain the grazing intake (Cangiano *et al.*, 2008) resulting to the low percentage intakes reported in the present study. Cangiano *et al.* (2008), described the mechanisms responsible for constraining the grazing intake of lucerne. The authors noted that animals usually graze lucerne forage by horizons, depending on the depth of each bite. During the morning grazing session of 4 hours, dairy cows consume about 50% of the available forage by volume from the top of the canopy, independent of the cattle's body weight. In subsequent grazing sessions, animals will consume about 50% of the available remaining forage, and quality will reduce significantly from top to bottom of the canopy as animals select the best parts of the plants first, and then the stemmy, fibrous portions of the plant at the bottom.

The consumption of maize and grass silage reduced the intake to 49% and 39 ± 1.07% respectively ($P < 0.001$). The effect of the supplements was also reflected in the reduction of grazing time by up to 7 minutes ($P < 0.001$), and the increase in time spent idling and lying ($P < 0.05$) compared to non-supplemented cows particularly towards the end of the four hours grazing session. This reduction in grazing time was consistent with observations from previous studies which reported that increasing the amount of supplement reduced grazing time by 8 to 20 minutes, but with little or no effect on biting rate (Arriaga-Jordan & Holmes, 1986; Bargo *et al.*, 2002; Bargo *et al.*, 2003; Gibb *et al.*, 2002; Kibon & Holmes, 1987; Rook *et al.*, 1994). The type of silage had no significant effect on grazing time ($P > 0.05$), an observation supported by previous study findings, which found that it was the amount, but not type of supplement (e.g. cereals and beet pulp) which reduced grazing time (by 8 to 12 min/kg of concentrate) for dairy cows grazing ryegrass (Kibon & Holmes, 1987). Supplements tend to influence grazing time and biting rate mainly through animal-related mechanisms such as short-term satiety signals like rumen physical fill and metabolic signals (Forbes, 2007; Rook, 2000). Thus, in the present study, the reduction in grazing time may have been due to the effect of silage supplements on physical fill of rumen (short-term physical regulation of intake) which may have in turn ceased grazing intake by up to 7 minutes ($P < 0.001$) compared to the grazing time for the non-supplemented cows during the morning grazing session of 4 hours.

5.3.6 Nitrogen concentration in milk, urine and faeces

5.3.6.1 N – intake, NUE and N - excretion

Cows offered lucerne only consumed higher amounts of lucerne N (530.78 ± 15.25 g N/cow/day; $P = 0.043$) compared to cows supplemented with maize and grass silage (479.43 and 484.72 ± 15.25 g N/cow/day respectively). The silage N increased the total N intake of supplemented cows, but which did not differ significantly ($P > 0.05$) from that of un-supplemented cows (Table 4.12). The total selected N intakes estimated from selected CP (362.57 ± 14.72 g/kg DM) were even higher but did not also differ ($P > 0.05$). The total selected N intakes were in the ranges of 820.52, 789.48 and 802.28 ± 23.58 g N/cow/day for cows offered lucerne only, lucerne + maize silage and lucerne + grass silage respectively. The nitrogen use efficiency (NUE) averaged at 18.8 ± 0.47% for all cows in the three dietary treatments, which can be classified as generally low compared to 25% typical for pasture grazed dairy cows (Huhtanen & Hristov, 2009; Kohn, Dinneen, & Russek-Cohen, 2005). Feeding maize or grass silage did not improve the NUE and microbial N flow. Similar observations on NUE have been published for dairy cows grazing grass pastures and offered supplemental carbohydrate (Kolver, Muller, Varga, & Cassidy, 1998a).

Castillo (1999), observed that for animals grazing high quality pastures (grass or lucerne), the high content and rumen availability of crude protein as well as the problems of controlling grazing behaviour, suggests it may be difficult to improve energy and protein interaction in the rumen by supplementation. Changes in rumen nitrogen degradation and ammonia concentration under grazing tend to be affected by grazing behaviour. The time spent grazing in a 24-hour period will be influenced by the food requirement of the animal, the amount and distribution of vegetation and by the rate (grams ingested per minute and per bite) at which the animals eat (Arnold, 1981), but a classical diurnal pattern of grazing is characterized by two period: each around 4-hours. This suggests that in each diurnal pattern, increasing quantities of fresh forages are consumed and degraded in a 4-hour period, producing a peak of rumen ammonia concentration in between 3 or 4-hours (Castillo & Gallardo, 1995; Kolver *et al.*, 1998a). Such changes plus seasonal changes and diurnal fluctuations of rumen metabolites (Beever & Siddons, 1986; Beever *et al.*, 1978; Kolver *et al.*, 1998a), make it difficult to obtain a high efficiency of nitrogen utilisation under grazing conditions.

Although an increase in total milk-solids was not observed on the supplementation of cows grazing lucerne pasture in autumn, clear benefits were seen in terms of urinary N excretion. Silage supplements effectively minimised urinary-N excretion and increased the content of faecal-N. The intake of maize and grass silage reduced (P = 0.010) urinary-N excretion to 365.28 and 352.52 ± 18.99 g/day respectively compared to 434.63 ± 18.99 g/day for lucerne only cows. When compared to control group, the supplements reduced urinary N output by 19% and 16% for cows fed grass and maize silage respectively. Cows consuming grass silage excreted slightly higher faecal N content (2.98% N) than those fed maize silage (2.83 %N), which was 9% and 4% higher than that for cows offered lucerne only. These N-output findings in the present study are consistent with those reported in previous studies which found feeding low protein feed such as maize or grass silage (7.5 and 15% CP) in conjunction with high protein pasture dilutes dietary protein content and reduces N excretion from grazing cows by up to 70% (Aarts *et al.*, 1999; Jarvis, Wilkins, & Pain, 1996; Kebreab, France, Beever, & Castillo, 2001; Ledgard, 2006; Ledgard *et al.*, 2000). Ledgard (2006), reported that feeding maize silage reduced the N content of urine by up to 70% and out of the total nitrogen excreted in the dung and urine, cows fed maize silage excreted a greater proportion in the dung while cows fed pasture excreted a greater proportion in the urine. However the quantity of N excreted in dung with a maize diet was relatively small.

This partitioning of nitrogen to urine and faeces by dairy cows can be explained from digestion mechanisms previously described, whereby it has been postulated that N excreted in faeces (g d^{-1}) originates from truly indigestible feed protein, undigested microbial N and endogenous N. Apparently indigestible N is only weakly dependent on the N content of forage (Demarquilly, Grenet, & Andrieu, 1981). For most diets fed to dairy cows, faecal N is directly related to the total DMI and averages 7.2 g N kg^{-1} DMI (Peyraud, Vérité, & Delaby, 1995). On the contrary, N excreted in urine (g d-1) is directly affected by the level and composition of the ingested protein. Increases in urinary N may originate from an excess of rumen-degradable N vs. rumen microbial requirements or from an excess or unbalanced amino-acid supply vs. cow requirements. Both lead to the production of urea that diffuses in the organism and is ultimately excreted in the urine, where it constitutes 10 to 80 % of urinary N (Peyraud *et al.*, 1995). Urea-N is rapidly converted to ammonia (NH_3) and easily volatilized or leached.

Cows offered lucerne only had the highest concentration of MUN (15.85 ± 0.39 mmol/L; $P = 0.044$) than those offered maize and grass silage (15 and 15.30 ± 0.39 mmol/L respectively). Excessive protein intake has been implicated to be a common nutritional factor responsible for such elevated MUN (Jonker, Kohn, & Erdman, 1998). Tyrrell, Moe, and Flatt (1970), demonstrated that if the high dietary intake is low in energy or high in protein to energy ratio, rumen bacteria will have reduced efficiency in utilizing free ammonia to synthesize protein, which can result in increased BUN or MUN (Broderick & Clayton, 1997; Hof, Vervoorn, Lenaers, & Tamminga, 1997; Rajala-Schultz & Saville, 2003). Cows in the present study consumed lucerne herbage with relatively higher CP (~36% CP), particularly the non-supplemented cows, which could have elevated the MUN levels up to 15.85 ± 0.39 mmol/L. Several overseas studies have reported MUN concentrations of 2.5 - 3.3 mmol/L (15-20 mg/dL) (Butler, 1998; Ferguson & Chalupa, 1989; Pehrson, Forshell, & Carlsson, 1992; Rajala-Schultz, Saville, Frazer, & Wittum, 2001), to cause negative effects on dairy cow fertility associated with long inter-oestrous interval due to low-progesterone concentration in the blood (Larson, Butler, & Currie, 1997), and decreased uterine pH, that could make the environment within the uterus unsuitable for early embryo development particularly at first service (Elrod, Van Amburgh, & Butler, 1993; Ferguson, Galligan, Blanchard, & Reeves, 1993; Guo, Russek-Cohen, Varner, & Kohn, 2004). However in New Zealand, relatively higher MUNs and BUNs of up to 40-50 mg/dL (7-8 mmol/L) have been reported as common in most studies with dairy cows fed ryegrass pastures (Harkin, 2013; Roche, Petch, & Kay, 2005; Smith *et al.,* 2001). Even higher MUN and BUN concentrations $\geq$ 60 mg/dL (10 mmol/L) have been recorded in studies with dairy cows grazing N-fertilised herbage (particularly in spring) (Ordonez *et al.*, 2007). This is three to four times greater than the reported safe level for reproduction for dairy cows in overseas. The MUN concentrations for lucerne fed cows in the present study (15 to 15.85 ± 0.39 mmol/L) are almost twice that for ryegrass fed cows in New Zealand and almost five times greater than the recommended overseas level. In spite of these high MUN concentrations, the pregnancy (foetal development) of cows in the current study was not affected, previous researches also show no potential negative effects of the high MUN levels on the reproduction of dairy cows in New Zealand (Ordóñez *et al.*, 2004; Ordonez *et al.*, 2007; Smith *et al.*, 2001). Although, it is not clear why New Zealand cows are able to tolerate such higher dietary crude protein (MUN and BUN) levels and still maintain a normal reproductive profile; it has been suggested that it may be because New Zealand cows are well adapted to high protein diets as a result of continued consumption overtime (Roche, 2010). Some authors have suggested that it could be due to the lack of relationship between high MUN/BUN or pasture crude protein content and if a cow conceives (gets pregnant) or not (Kenny, Boland, Diskin, & Sreenan, 2001, 2002; Ordonez *et al.*, 2007)

5.3.6.2 Non-urea components in urine

Dietary treatments had no effect on urinary concentrations of creatinine, purine derivatives (PD) i.e. allantoin and uric acid, calculated PD index and the total microbial N supply ($P > 0.05$). However, the period effect was significant ($P < 0.001$). PD index is a relative measure of intestinal microbial protein supply (Chen, Mejia, Kyle, & Ørskov, 1995), and the lack of difference in the PD index indicates similar levels of microbial activity between the dietary treatments. Lack of variation in PD index was also reported for dairy cows grazing simple and diverse pastures

(Totty, Greenwood, Bryant, & Edwards, 2012). In the present study, the estimated microbial N supply was relatively higher than the total N intake from the general lucerne herbage on offer, but lower than the total selected N intakes estimated from selected CP (Table 4.12). This suggests that cows may have actually consumed higher amounts of total N than those estimated from the CP content in the general pasture; hence a more accurate alternative method could have been used to quantify the total N-intake.

Urinary PD comprised of allantoin = 89.12%, uric acid = 10.02%, xanthine and hypoxanthine = 0.86%. The higher allantoin content of the total urinary PD excreted corroborates with the findings of Gonda and Lindberg (1994), Shingfield and Offer (1998) and Totty *et al.* (2012), which found concentrations above 90%. No significant amounts of xanthine and hypoxanthine (0.86%) were detected. The absence of salvageable PD - xanthine and hypoxanthine in the urine samples confirmed the high ability of the cows to oxidise absorbed purine bases to non-re utilisable PD (Balcells, Parker, & Seal, 1992; Chen, Hovell, Ørskov, & Brown, 1990; Tas & Susenbeth, 2007). The excretion of creatinine was relatively constant across the three dietary treatments ($P > 0.05$), an observation that follows the suggestion that the excretion rate of creatinine is relatively constant in healthy animals and remains independent of level of intake (Chen & Gomes, 1995). Moreover, the use of creatinine as an internal marker of urinary output relies on the assumption that the creatinine excretion through urine is affected neither by diet nor the physiological status of the animal, but is excreted in proportion to body weight. There was little difference in the liveweight of the cows used in this study ($P > 0.05$), and hence they had almost similar excretion of urinary creatinine.

5.3.7 General discussion on grazed lucerne & supplements in dairy systems

The traditional place of lucerne in farm enterprises has been special purpose stands grown for conservation or fattening lambs. Consequently, little research has been conducted in New Zealand on the use of lucerne for dairy production (Bryant, 1978; Douglas, 1986; Moot, 2009). Overseas, lucerne is used as a high quality feed for dairy cows, particularly under cut and carry and preserved feed systems, although to achieve high production, energy supplementation is needed (Barnes & Gordon, 1972; Basigalup & Ustarroz, 2007; Cangiano *et al.*, 2008). Previous studies have shown that direct grazing of lucerne can play an important role in reducing operative costs and decreasing quality loss due to forage conservation (hay or silage) (Basigalup & Ustarroz, 2007; Iversen, 1967), and farmers' experiences indicate that it is possible to reach high animal response under direct grazing provided appropriate management practices are utilised. Findings from this study also showed that lucerne can be directly grazed by mid-lactating dairy cows in autumn, and maize or grass silage supplement can be offered when the regrowth of lucerne decreases to sustain high DM and ME intakes. In addition, the economic benefits of a lucerne system are potentially very high. Using Lincoln University Dairy Farm data shows increases of 38% and higher, in cash operating surplus, with little increase in total expenditure (Campion, 2011).

The typical growth and development of lucerne are strongly linked and altered by environmental signals (i.e. temperatures and photoperiods) across seasons. These in-turn regulate the remobilisation of carbohydrate and amino acid reserves from stores in the crown and tap root to new basal buds located on the crown of the plant (Moot *et al.*, 2003). Thus, forage accumulation rates (mass per unit area per day) in most parts of New Zealand are highest in spring to early summer (October – February) until high temperatures become limiting, slower in early spring and autumn (September and March–May) and very slow in winter (June–August) when temperature and photoperiod are lowest (Cangiano *et al.*, 2008; Moot *et al.*, 2003). Additionally, lucerne variety, fertility, irrigation and other factors affect growth rates. Therefore, careful grazing management is critical to obtain high yields. Each season has certain requirements (Moot, Avery, & Avery, 2009). The appropriate grazing management practices for lucerne based pastures per season have been detailed by Moot *et al.* (2003). The management differs from ryegrass based pastures because emerging shoots from the crown require protection, and the lucerne stand must fully flower once a year to restore root reserves. To optimise the use of lucerne for dairy production, rotational grazing is widely recommended as the best management practice (Basigalup & Ustarroz, 2007; Brownlee, 1973; Cangiano *et al.*, 2008; Knight, 1987; Lodge, 1991; Moot *et al.*, 2003; Pecetti, Romani, & Piano, 2006; Sewell, Hill, & Reich, 2011; Teixeira, Moot, Brown, & Fletcher, 2007; White & Cosgrove, 1990), and is integral for ensuring persistence of the stand (McGowan, Sheath, Webby, & Moot, 2003; Milne, 2011). In the present study, optimal management of lucerne herbage was achieved under rotational grazing. Cows were grazed in four paddocks each of 1.5 ha, for 15 days followed by a rotational length of 30 days that allowed for the regrowth of lucerne. Previous studies have demonstrated successful rotational grazing management for lucerne should have a resting period/rotational length of at least 35 days to allow for the recovery of the lucerne before subsequent defoliation (Avery, Avery, Ogle, Wills, & Moot, 2008; Basigalup & Ustarroz, 2007; Cangiano *et al.*, 2008). This rest period can be adjusted according to season, lucerne variety and level of supplement, with the aim of maintaining high forage value and avoiding defoliation of the new regrowth emerging from the crown. A typical rotational grazing program for lucerne-based dairy farms should entail division of the field into paddocks (≤ 8) of appropriate size (≥ 1 acre), and control of dairy cows, often by use of temporary fencing or use of existing fields in a system of pastures. The basic principle is managing cows for defoliation at specific plant maturity (grazing period), followed by a rest period before subsequent defoliation (Cangiano *et al.*, 2008). In a rotational stocking program, a grazing period of 7 to 10 days with at least 35 days of rest is considered safe for lucerne pasture and adequate for dairy cow production (Avery *et al.*, 2008; Campion, 2011; Cangiano *et al.*, 2008). The rotational grazing systems are mostly labour intensive, and when moderately stocked, result in lower harvesting efficiency per hectare but higher milk production compared to continuous grazing systems (Cangiano *et al.*, 2008).

With appropriate management, the potential for utilising lucerne forage in a dairy farm is high. In addition to the ability to produce more dry matter than conventional pasture per annum, lucerne has the ability to grow without nitrogen inputs, its resistance to grass grub, and has high feed value (Campion, 2011; Douglas, 1986; Mace, 1982). Furthermore, comparative studies conducted on the performance of dairy cows grazing lucerne vs. ryegrass based pastures (Baudracco *et al.*, 2011; Bryant, 1978; Conrad, Van Keuren, & Dehority, 1983; Limon Sanchez & Campling, 1982; Smith *et al.*, 2013; Woodward *et al.*, 2010), show that the performance (DMI, MEI and MS yield) of dairy cows under lucerne-based dairy systems is almost equal to or slightly higher compared to ryegrass-based dairy systems when the growth of lucerne is not limited. However, when the regrowth of lucerne is limited in late autumn and early spring, supplementary feeds (preferably conserved feed e.g. silage or hay) can be offered. And as demonstrated by the findings in the present study and from previous studies, the supplements increase the total DMI and MEI (White, 1982); reduce nitrogen excretion through urine (Aarts *et al.*, 1999; Jarvis *et al.*, 1996;

Kebreab *et al.*, 2001; Ledgard, 2006; Ledgard *et al.*, 2000); and enable substitution of the lucerne (Castillo *et al.*, 2001a), while maintaining milksolids production, milk yield and liveweight gain in mid to late lactation (Stockdale, Callaghan, & Trigg, 1987; Stockdale & Trigg, 1989). Although, there is need to further investigate lucerne management, and improve its production, through research and experience in the dairy situation.

5.3.8 Conclusions

The findings of this study show that feeding maize or grass silage to mid-late lactation dairy cows grazing autumn lucerne significantly increased total DMI and MEI; however cow responses to the supplements in terms of milk-solids yield, subR, MMR, FCE, LWT gain and BCs were generally low. This therefore implies that supplementary maize or grass silage feeds can be used in autumn to sustain high DM and ME intakes, and enable substitution of the sown lucerne crop to increase herbage cover on the farm during periods of reduced growth in autumn/winter and early spring. The carryover effects of substituted lucerne pasture and spared cow condition could also be turned to milksolids after calving in spring.

The results of this study also indicate that feeding either maize silage or grass silage to dairy cows in mid-lactation grazing in autumn lucerne has the potential to reduce nitrogen excretion through urine. The results showed that the silage supplements did not compromise milksolids production, milk yield and liveweight gain in mid lactation, and substitution rate was low. This further demonstrated the use of either maize or grass silage as a mitigation strategy to reduce the environmental impact of dairying in lucerne-based systems.

5.3.9 Recommendations

Based on the results from this study, it would be appropriate to recommend the restriction of lucerne allowance when supplements are fed, (e.g. reducing lucerne allowance from 15 to 12 kg DM/cow/day when 3 kg DM/cow/day of maize or grass silage supplements are fed). This would improve the utilisation of lucerne per hectare, reduce the N-intake (particularly from lucerne herbage), and maintain milksolids production, milk yield and liveweight gain in mid lactation.

Additionally, the data from this study and from literature represent an important source of information for future investigations to quantify the effects of supplement allowance and season on animal responses, and N-utilization by dairy cows grazing lucerne, in addition to evaluating the dynamics of rumen fermentation.

Appendix A.
Methods and Measurements

Table A. 5.1: Average milksolids, liveweight and age which were used to allocate cows randomly to the dietary treatments in period I and II of the experiment**.**

Period I			
Treatment	Age (yrs.)	LWT (kg)	MS (kg/d)
Lucerne	5.33	476	1.32
Lucerne + Maize silage	5.02	460	1.33
Lucerne + Grass silage	6.03	460	1.31
Grand mean	**5.46**	**465.33**	**1.32**
Period II			
Treatment	Age (yrs.)	LWT (kg)	MS (kg/d)
Lucerne	5.34	460	1.32
Lucerne + Maize silage	5.92	468	1.32
Lucerne + Grass silage	5.12	467	1.33
Grand mean	**5.46**	**465.00**	**1.32**

Table A. 5.2: Calibration heights (H1 – H5; cm), average height (cm), sample DM (g) and DM yield per ha that were used to derive the linear calibration equation (Figure 4.1).

Paddock	**Calibration**	**H_1**	**H_2**	**H_3**	**H_4**	**H_5**	**Av. Height**	**DM grams**	**KgDM/ha**
A2	1	50	49	49	44	47	47.80	57.80	2890
A2	2	51	45	42	47	45	46.00	72.10	3605
A2	3	48	48	47	44	46	46.60	59.20	2960
A2	4	53	37	50	52	44	47.20	31.70	1585
A2	5	51	49	48	49	51	49.60	58.40	2920
A5	6	26	29	26	29	30	28.00	33.40	1670
A5	7	16	13	14	15	15	14.60	26.70	1335
A5	8	40	47	38	45	48	43.60	50.80	2540
A5	9	22	21	18	16	20	19.40	26.70	1335
A5	10	25	34	31	31	28	29.80	38.10	1905
A4	11	40	43	50	41	42	43.20	47.40	2370
A4	12	44	30	30	32	24	32.00	45.40	2270
A4	13	33	24	30	40	30	31.40	43.90	2195
A4	14	26	20	26	18	20	22.00	15.90	795
A4	15	24	21	28	26	20	23.80	22.50	1125
A4	16	9	8	9	7	7	8.00	5.90	295
A7	17	37	35	35	33	36	35.20	42.40	2120
A7	18	19	21	18	16	18	18.40	22.80	1140
A7	19	57	45	50	54	60	53.20	62.60	3130
A7	20	51	50	47	47	47	48.40	49.00	2450
A7	21	51	52	53	45	57	51.60	65.70	3285

Appendix B.
Lab-work

Figure B. 5.1: Freeze-dried samples being ground to 1 mm for proximate analysis using wet chemistry procedures to determine content of NDF, ADF, soluble carbohydrates, ME, protein and dry matter digestibility (DMD).

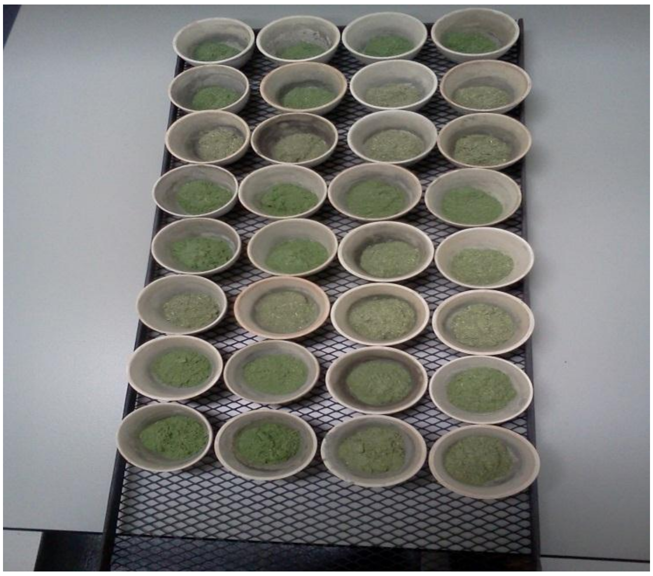


Figure B. 5.2: Ground samples in porcelain lab dishes which were used to determine ash by subsequent combustion in a muffle furnace (Model LAB 3A KW 5000 W, Max T: 1000 0C), at 550^0C for 4 hours.

References

Aarts, H. F. M., Habekotte, B., Hilhorst, G. J., Koskamp, G. J., Van Der Schans, F. C., & De Vries, C. K. (1999). Efficient resource management in dairy farming on sandy soil. *NJAS wageningen journal of life sciences*, *47*(2), 153-167.

Alder, F. E., & Minson, D. J. (1963). The herbage intake of cattle grazing lucerne and orchardgrass pastures. *Journal of agricultural science Cambridge University press 60*, 359–369.

Alderman, G. (1983). Supplementary feeds and forage interactions and their effects upon dairy cow performance.

ANKOM, Technology. (2010). Neutral Detergent Fiber in Feeds Filter Bag Technique (For A200, A200I). *Analytical Procedures.* Retrieved 05/10/2013, from http://www.ankom.com/analytical-procedures.aspx

AOAC, Association of Official Analytical Chemists. (1990). Fiber (Acid Detergent) and Lignin in Animal Feed (973.18) Official Methods of Analysis, 1990. *Association of Official Analytical Chemists*(15th Edition).

ARC. (1980). *Agricultural Research Council, The nutrient requirements of ruminant livestock*. Farnham Royal, UK: Commonwealth Agricultural Bureaux.

Armentano, L. E., Bertics, S. J., & Riesterer, J. (1993). Lack of Response to Addition of Degradable Protein to a Low Protein Diet Fed to Midlactation Dairy Cows. *Journal of Dairy Science, 76*(12), 3755-3762. doi: http://dx.doi.org/10.3168/jds.S0022-0302(93)77718-8

Arnold, G.W. (1981). Grazing behaviour. In F.H.W. Morley (Ed.), *World Animal Science, B1.Grazing Animals* (pp. 79-104). Oxford, UK.: Elsevier Scientific Publishing Company.

Arriaga-Jordan, C. M., & Holmes, W. (1986). The effect of concentrate supplementation on high-yielding dairy cows under two systems of grazing. *Journal of Agricultural Science, Cambridge, 107*(2), 453-461.

Avery, D., Avery, F., Ogle, G. I., Wills, B. J., & Moot, D. J. (2008). Adapting farm systems to a drier future. *Proceedings of the New Zealand Grassland Association, 70*, 13–18.

Balcells, J., Parker, D. S., & Seal, C. J. (1992). Purine metabolite concentrations in portal and peripheral blood of steers, sheep and rats. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 101*(4), 633-636.

Bao, J., Giller, P. S., & Kett, J. J. (1992). The effect of milk production level on grazing behaviour of Friesian cows under variable pasture conditions. *Irish Journal of Agricultural and Food Research*, 23-33.

Bargo, F., Muller, L. D., Delahoy, J. E., & Cassidy, T. W. (2002). Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. *Journal of Dairy Science*, *85*(7), 1777-1792.

Bargo, F., Muller, L. D., Kolver, E. S., & Delahoy, J. E. (2003). Invited Review: Production and Digestion of Supplemented Dairy Cows on Pasture. *Journal of Dairy Science, 86*(1), 1-42.

Barnes, R. F., & Gordon, C. H. (1972). Feeding Value and On-farm Feeding. In C. H. Hanson (Ed.), *Alfalfa Science and Technology* (pp. 601-630): American Society of Agronomy.

Bartels, H., & Böhmer, M. (1971). Micro-determination of creatinine. *Clinica chimica acta; international journal of clinical chemistry, 32*(1), 81.

Basigalup, D.H., & Ustarroz, E. (2007). *Grazing alfalfa systems in the Argentinean Pampas*. Paper presented at the 37th Calfornia Alfalfa and Forage Symposium Monterey, Calfornia. http://alfalfa.ucdavis.edu/+symposium/proceedings/2007/07-51.pdf

Baudracco, J., Lopez-Villalobos, N., Holmes, C. W., & Macdonald, K. A. (2010). Effects of stocking rate, supplementation, genotype and their interactions on grazing dairy systems: a review. *New Zealand Journal of Agricultural Research, 53*(2), 109-133.

Baudracco, J., Lopez-Villalobos, N., Romero, L. A., Scandolo, D., Maciel, M., Comeron, E. A., . . . Barry, T. N. (2011). Effects of stocking rate on pasture production, milk production and reproduction of supplemented crossbred Holstein–Jersey dairy cows grazing lucerne pasture. *Animal Feed Science and Technology, 168*(1–2), 131-143. doi: http://dx.doi.org/10.1016/j.anifeedsci.2011.03.017

Beever, D. E., & Doyle, P. T. (2007). Feed conversion efficiency as a key determinant of dairy herd performance: a review. *Animal Production Science, 47*(6), 645-657.

Beever, D.E., & Reynolds, C.K. (1994). *Forage quality, feeding value and animal performance.* Paper presented at the Grassland and society: proceedings of the 15th general meeting of the European Grassland Federation.

Beever, D.E., & Siddons, R.C. (1986). Digestion and metabolism in the grazing ruminant. In L.P. Milligan, W.L. Grovum & A. Dobson (Eds.), *Proceedings VIth International Symposium of Ruminant Physiology* (pp. 479-497). Englewood, New Jersey.

Beever, D.E., Terry, R.A., Cammell, S.B., & Wallace, Annie S. (1978). The digestion of spring and autumn harvested perennial ryegrass by sheep. *The Journal of Agricultural Science, Cambridge, 90*(03), 463-470.

Black, A. D. (2004). *A comparison of Caucasian and white clovers in temperate pastures.* (Ph.D Thesis), Lincoln University, Canterbury, New Zealand.

Blaser, R. E. (1982). Integrated pasture and animal management. *Tropical Grasslands, 16*.

Bretschneider, G., Peralta, M., Santini, F. J., Fay, J. P., & Faverin, C. (2007). Influence of corn silage supplementation before alfalfa grazing on ruminal environment in relation to the occurrence of frothy bloat in cattle. *Animal feed science and technology, 136*(1), 23-37.

Broderick, Glen. A., & Clayton, Murray. K. (1997). A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *Journal of dairy science, 80*(11), 2964-2971.

Broster, William. H., & Broster, Valerie. J. (1998). Body score of dairy cows. *Journal of Dairy Research, 65*(01), 155-173.

Brown, H. E., & Moot, D. J. (2004). Quality and quantity of chicory, lucerne, and red clover production under irrigation. *Proceeding of the New Zealand Grasslands association, 66*, 257-264.

Brown, H. E., Moot, D. J., & Pollock, K. M. (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*(4), 423-439. doi: 10.1080/00288233.2005.9513677

Brown, H. E., Moot, D. J., Pollock, K. M., & Inch, C. (2000). *Dry matter production of irrigated chicory, lucerne and red clover in Canterbury.* Paper presented at the Proceedings of the Thirtieth Annual Conference, Agronomy Society of New Zealand, Palmerston North, New Zealand, 2000.

Brown, H. E., Moot, D. J., & Pollock, Keith M. (2003). Long term growth rates and water extraction patterns of dryland chicory, lucerne and red clover. *In:* D.J. Moot (ed). Legumes for dryland pastures. *Proceeding of the New Zealand Grasslands association*.

Brownlee, H. (1973). Effects of four grazing management systems on the production and persistence of dryland lucerne in central western New South Wales. *Animal Production Science, 13*(62), 259-262.

Bryant, A. M. (1978). Milk yield and composition from cows grazing lucerne. *Proceeding of the New Zealand Grasslands association, 38*, 185-190.

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

Burke, J. L. (2004). *Determination of digestion parameters to develop and evaluate forage mixtures for pasture-fed ruminants.* Ph. D. thesis, Massey University, Palmerston North, New Zealand.

Burke, J.L., Waghorn, G.C., & Chaves, A.V. (2002). *Improving animal performance using forage-based diets.* Paper presented at the Proceedings - New Zealand Society of Animal Production.

Butler, W. R. (1998). Review: effect of protein nutrition on ovarian and uterine physiology in dairy cattle. *Journal of dairy science, 81*(9), 2533-2539.

Campion, R. (2011). *Utilising lucernes potential for dairy farming*. Paper presented at the 18th International Farm Managment Congress, Methven, Canterbury, New Zealand. http://www.ifmaonline.org/pdf/congress/11_NPR_Campion_P227-235.pdf

Cangiano, C. A., Castillo, A. R. , Guerrero, J. , & Putnam, D. H. . (2008). Alfalfa Grazing Management. In C. G. Summers & D. H. Putnam (Eds.), *Irrigated alfalfa management in Mediterranean and Desert zones.* (Vol. Publication 8304.). Oakland USA: University of California Agriculture and Natural Resources.

Castillo, A. R., & Gallardo, M. . (1995). Dairy cows supplementation on alfalfa pastures, concentrates and conserved forages. In Spanish (Ed.), *La alfalfa en la Argentina* (pp. 198-204.). San Juan. Argentina.: INTA. National Institute of Agricultural Technology.

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

Castillo, A. R., Romero, Luis Alberto, Quaino, Oscar Rodolfo, Comerón, Eduardo Alberto, & Gaggiotti, Mónica del Carmen. (2001a). *Supplementation of dairy cows under alfalfa grazing conditions with ground corn.* Paper presented at the International Grassland Congress. 19. 2001, de febrero, 2001. Sao Pedro, Sao Pablo. BR., Rafaela Santa Fe Argentina.

Castillo, A.R., Kebreab, E., Beever, D.E., Barbi, J.H., Sutton, J.D., Kirby, H.C., & France, J. (2001b). The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *Journal of Animal Science, 79*(1), 247-253.

Castillo, Alejandro R. (1999). *Improving nitrogen utilisation in dairy cows.* (Doctor of Philosophy), The University of Reading.

Chacon, E., Stobbs, T. H., & Sandland, R. L. (1976). Estimation of herbage consumption by grazing cattle using measurements of eating behaviour. *Grass and Forage Science, 31*(2), 81-87.

Champion, R. A., Rutter, S. M., & Delagarde, R. (1998). *Recent developments with the IGER Behaviour Recorder.* Paper presented at the Proc. IXth European Intake Workshop. IGER, North Wyke, UK.

Charlton, D., & Stewart, A. (2000). Pasture and forage plants for New Zealand. Grassland Research and Practice. *New Zealand Grassland Association, 8*, 74.

Chatepa, Lesten Eliez Chisomo. (2012). *The growing and feeding of lucerne,(Medicago sativa sativa), to dairy cows.* (Master of Philosophy), University of Glasgow.

Chen, X. B., Hovell, F. D., Ørskov, E. R., & Brown, D. S. (1990). Excretion of purine derivatives by ruminants: effect of exogenous nucleic acid supply on purine derivative excretion by sheep. *British Journal of Nutrition, 63*(01), 131-142.

Chen, X. B., Mejia, A. T., Kyle, D. J., & Ørskov, E. R. (1995). Evaluation of the use of the purine derivative: creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants: studies in sheep. *The Journal of Agricultural Science, 125*(01), 137-143. doi: doi:10.1017/S002185960007458X

Chen, Xubin B., & Gomes, M. J. (1995). *Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives-an overview of the technical details*: International Feed Resources Unit.

Christensen, R.A., Lynch, G.L., Clark, J.H., & Yu, Y. (1993). Influence of amount and degradability of protein on production of milk and milk components by lactating Holstein cows. *Journal of dairy science, 76*(11), 3490-3496.

Clark, D. A. (1993). Silage for milk production *Proceedings of Ruakura dairy farmers' conference 45* (pp. 41-46). Hamilton: Dairy Research Corporation.

Clark, D. A. (1995). *Summer milk-pasture and crops.* Paper presented at the Proceedings of the Ruakura Dairy Farmers' Conference.

Clark, D. A., Lambert, M. G., Rolston, M. P., & Dymock, N. (1982). Diet selection by goats and sheep on hill country. *Proceedings of the New Zealand Society of Animal Production, 42*, 155-157.

Clarke, R.T.J., & Reid, C.S.W. (1974). Foamy bloat of cattle. A review. *Journal of Dairy Science, 57*, 753-785.

Cohen, D. C., Stockdale, C. R., & Doyle, P. T. (2006). Feeding an energy supplement with white clover silage improves rumen fermentation, metabolisable protein utilisation, and milk production in dairy cows. *Crop and Pasture Science, 57*(4), 367-375.

Conrad, H.R., Van Keuren, R.W., & Dehority, B.A. (1983). *Top-grazing high-protein forages with lactating cows [Alfalfa]*. Paper presented at the Proceedings Fourteenth International Grassland Congress., Lexington.

Cook, B.G., Pengelly, B.C., Brown, S.D., Donnelly, J.L., Eagles, D.A., Franco, M.A., . . . Peters, M. (2005). Tropical Forages: an interactive selection tool,[CD-ROM]. *CSIRO, DPI&F (Qld), CIAT and ILRI, Brisbane, Australia. , 14*, 2006.

Cosgrove, G.P., & Edwards, G.R. (2007). Control of grazing intake. In 'Pasture and supplements for grazing animals'.(Eds PV Rattray, IM Brooks, AM Nicol) pp. 61–80. *New Zealand Society of Animal Production: Hamilton*.

Czauderna, M., & Kowalczyk, J. (2000). Quantification of allantoin, uric acid, xanthine and hypoxanthine in ovine urine by high-performance liquid chromatography and photodiode array detection. *Journal of Chromatography B: Biomedical Sciences and Applications, 744*(1), 129-138.

DairyNZ. (2012). Feed requirements of milking cows. *DairyNZ FarmFact, 1*, 1-7. www.dairynz.co.nz/file/fileid/36297

Danelon, J. L., Locatelli, M. L., Gallardo, M., & Guaita, S. (2002). Herbage intake and ruminal digestion of alfalfa: a comparison between strip and zero grazed dairy cows. *Livestock production science, 74*(1), 79-91.

de Klerk, Howard. (2012). Milk Response to grain as achieved by New Zealand pasture based farmers. In DairyNZ (Ed.). South Island, New Zealand: South Island Dairy Event, New Zealand.

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. In P. V. Rattray, I. M. Brookes & A. M. Nicol (Eds.), *Pastures and supplements for grazing animals. Occasional publication no. 14.* (Vol. 14, pp. 97-115). Hamilton, New Zealand: New Zealand Society of Animal Production.

Delagarde, R., Peyraud, J.L., & Delaby, L. (1997). The effect of nitrogen fertilization level and protein supplementation on herbage intake, feeding behaviour and digestion in grazing dairy cows. *Animal Feed Science and Technology, 66*(1), 165-180.

Demarquilly, C., Grenet, E., & Andrieu, J. (1981). Les constituants azotés des fourrages et la prévision de la valeur azotée des fourrages. *Prévision de la valeur nutritive des aliments des ruminants. INRA Publications, Versailles*, 129-154.

Dewhurst, R. J., Davies, L. J., & Kim, E. J. (2010). Effects of mixtures of red clover and maize silages on the partitioning of dietary nitrogen between milk and urine by dairy cows.

Dewhurst, R. J., Delaby, L., Moloney, A., Boland, T., & Lewis, E. (2009). Nutritive value of forage legumes used for grazing and silage. *Irish Journal of Agricultural and Food Research, 48*(2), 167-187.

Dewhurst, R. J., Fisher, W. J., Tweed, J. K. S., & Wilkins, R. J. (2003). Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. *Journal of Dairy Science, 86*(8), 2598-2611.

Dewhurst, R. J., Merry, R. J., & Davies, L. J. . (2005). *Effects of mixtures of red clover and maize silages on milk production and nitrogen utilisation by dairy cows.* . Paper presented at the Proceedings of the British Society of Animal Science Annual Meeting: 23.

Dewhurst, R.J. (2006). *Manipulating cow diets to reduce nutrient wastage to the environment*. Paper presented at the South Island Dairy Event (SIDE), Stadium Southland, Invercargill, New Zealand.

Dhiman, T.R., Cadorniga, C., & Satter, L.D. (1993). Protein and energy supplementation of high alfalfa silage diets during early lactation. *Journal of dairy science, 76*(7), 1945-1959.

Dijkstra, J. , France, J. , Ellis, J. L. , Kebreab, E., Lopez, S., Rejis, J. , & Bannink, A. . (2009). Effects of nutritional strategies on N and methane losses in dairy cattle. ‘Modelling nutrient digestion and utilization in farm animals' *Proceedings of the 7th international workshop MODNUT* (pp. 67). Paris.

Dillon, P. (2007). Achieving high dry-matter intake from pasture with grazing dairy cows. *Frontis, 18*, 1-26.

Dougherty, C. T., Lauriault, L. M., Cornelius, P. L., & Bradley, N. W. (1987). Herbage intake rates of beef cattle grazing alfalfa. *Agronomy journal, 79*(6), 1003-1008.

Dougherty, C. T., Lauriault, L. M., Cornelius, P. L., & Bradley, N. W. (1989a). Accessibility of herbage allowance and ingestive behavior of beef cattle. *Applied Animal Behaviour Science, 23*(1), 87-97.

Dougherty, C. T., Lauriault, L. M., Cornelius, P. L., & Bradley, N. W. (1989b). Herbage allowance and intake of cattle. *The Journal of Agricultural Science Cambridge Univ. Press, 112*(03), 395-401.

Dougherty, C. T., Lauriault, L. M., Cornelius, P. L., & Bradley, N. W. (1989c). Short-term fasts and the ingestive behaviour of grazing cattle. *Grass and Forage Science, 44*(3), 295-302.

Dougherty, C. T., Smith, E. M., Bradley, N. W., Forbes, T. D. A., Cornelius, P. L., Lauriault, L. M., & Arnold, C. D. (1988). Ingestive behaviour of beef cattle grazing alfalfa (*Medicago sativa* L.). *Grass and Forage Science, 43*(2), 121-130.

Douglas, J. A. (1986). The production and utilization of lucerne in New Zealand. *Grass and Forage Science, 41*(2), 81-128. doi: 10.1111/j.1365-2494.1986.tb01796.x

Edwards, G. R, Parsons, A. J, & Rasmussen, S. (2007). High sugar ryegrasses for dairy systems. In D.F Chapman, D.A Clark, K.L MacMillan & D.P Nation (Eds.), *Meeting the Challenges for Pasture-Based Dairying. Proceedings of the Australasian Dairy Science Symposium* (pp. 307–334). Melbourne, Australia: University of Melbourne, National Dairy Alliance.

Elrod, C. C., Van Amburgh, M., & Butler, W. R. (1993). Alterations of pH in response to increased dietary protein in cattle are unique to the uterus. *Journal of Animal Science, 71*(3), 702-706.

Ferguson, James D., & Chalupa, William. (1989). Impact of Protein Nutrition on Reproduction in Dairy Cows. *Journal of dairy science, 72*(3), 746-766.

Ferguson, James D., Galligan, David T., Blanchard, Terry, & Reeves, Matthew. (1993). Serum urea nitrogen and conception rate: the usefulness of test information. *Journal of dairy science, 76*(12), 3742-3746.

Fletcher, L. R. (1976). Effect of season and regrowth period on the in vitro digestibility of irrigated lucerne in Canterbury. *New Zealand Journal of Experimental Agriculture, 4*(4), 469-471.

Forbes, John Michael. (2007). *Voluntary food intake and diet selection in farm animals*: CABI.

Forbes, T. D. (1988). Researching the plant-animal interface: the investigation of ingestive behavior in grazing animals. *Journal of Animal Science, 66*(9), 2369.

George, S.K., Dipu, M.T., Mehra, U.R., Singh, P., Verma, A.K., & Ramgaokar, J.S. (2006). Improved HPLC method for the simultaneous determination of allantoin, uric acid and creatinine in cattle urine. *Journal of Chromatography B, 832*(1), 134-137.

Gibb, M. J., Huckle, C. A., & Nuthall, R. (2002). Effects of level of concentrate supplementation on grazing behaviour and performance by lactating dairy cows grazing continuously stocked grass swards. *Animal Science (Penicuik, Scotland), 74*(2), 319-335.

Gibb, M. J., Huckle, C. A., Nuthall, R., & Rook, A. J. (1997). Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. *Grass and Forage Science, 52*(3), 309-321.

Givens, D. I., Everington, Jeannie M., & Adamson, A. H. (1989). The digestibility and metabolisable energy content of grass silage and their prediction from laboratory measurements. *Animal Feed Science and Technology, 24*(1–2), 27-43. doi: http://dx.doi.org/10.1016/0377-8401(89)90018-7

Givens, D.I. (1989). Predicting the metabolisable energy content of high temperature dried grass and lucerne. *WF Raymond Proceedings of Dri-Crop, 89*, 117-121.

Givens, D.I. (1990). *The digestibility of maize silage–an update.* Paper presented at the Proceedings of the Second Annual Conference of the Maize Growers Association, Royal Agricultural College, Cirencester.

Gonda, Horacio Leandro, & Lindberg, Jan Erik. (1994). Evaluation of Dietary Nitrogen Utilization in Dairy Cows Based on Urea Concentrations in Blood, Urine and Milk, and on Urinary Concentration of Purine Derivatives. *Acta Agriculturae Scandinavica, Section A – Animal Science, 44*(4), 236-245. doi: 10.1080/09064709409410904

Grainger, C. (1990). Effect of stage of lactation and feeding level on milk yield response by stall-fed dairy cows to change in pasture intake. *Animal Production Science, 30*(4), 495-501.

Grainger, C., & Mathews, G.L. (1989). Positive relation between substitution rate and pasture allowance for cows receiving concentrates. *Australian Journal of Experimental Agriculture, 29*(3), 355-360. doi: http://dx.doi.org/10.1071/EA9890355

Gregorini, P. , Beukes, P. , Bryant, R. H. , & Romera, A. . (2010). A brief overview and simulation of the effects of some feeding strategies on nitrogen excretion and enteric methane emission from grazing dairy cows. In G.R. Edwards & R.H. Bryant (Eds.), *'Meeting the challenges for pasture- based dairying' Proceedings of the 4th Australasian Dairy Science Symposium. (This proceedings)* (pp. 29-43).

Gregorini, P., Clark, C. E. F., Jago, J. G., Glassey, C. B., McLeod, K. L. M., & Romera, A. J. (2009). Restricting time at pasture: Effects on dairy cow herbage intake, foraging behavior, hunger-related hormones, and metabolite concentration during the first grazing session. *Journal of Dairy Science, 92*(9), 4572-4580. doi: http://dx.doi.org/10.3168/jds.2009-2322

Grovum, W. L., & Chapman, H. W. (1988). Factors affecting the voluntary intake of food by sheep. 4. The effect of additives representing the primary tastes on sham intakes by oesophageal-fistulated sheep. *The British journal of nutrition, 59*(1), 63.

Guo, Kejun, Russek-Cohen, E, Varner, M. A., & Kohn, R. A. (2004). Effects of milk urea nitrogen and other factors on probability of conception of dairy cows. *Journal of dairy science, 87*(6), 1878-1885.

Guy, M. C., Watkin, B. R., & Clark, D. A. (1981). Effects of season, stocking rate, and grazing duration on diet selected by hoggets grazing mixed grass-clover pastures. *New Zealand journal of experimental agriculture, 9*(2), 141-146.

Hacker, J. B., & Minson, D. J. (1981). The digestibility of plant parts. *Herbage abstracts, 51*(9), 459-482.

Hancock, J., & McMeekan, C. P. (1954). Studies of grazing behaviour in relation to grassland management: III. Rotational compared with continuous grazing. *The Journal of Agricultural Science, 45*(01), 96-103.

Harkin, Conal Joseph. (2013). *Supplementation of dairy cows grazing to low and high post grazing pasture height.* (Master of Agricultural Science), Lincoln University, 2013. Retrieved from http://hdl.handle.net/10182/5962

Harris, S. L., Auldist, M. J., Clark, D. A., & Jansen, E. B. L. (1998). 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.

Harris, S. L., Clark, D. A. , & Laboyrie, P. J. (1998). Birdsfoot trefoil – an alternative legume for New Zealand dairy systems. *Proceedings of the New Zealand Grassland Association, 60*, 99–103.

Harris, S. L., Clark, D. A., Auldist, M. J., Waugh, C. D., & Laboyrie, P. G. (1997). Optimum white clover content for dairy pastures. *Proceedings of the New Zealand Grassland Association, 59*, 29–33.

Hart, R. H. (1972). Forage yield, stocking rate, and beef gains on pasture. *Herbal Abstracts, 42*, 345-353.

Hart, R. H., Samuel, M. J., Test, P. S., & Smith, M. A. (1988). Cattle, vegetation, and economic responses to grazing systems and grazing pressure. *Journal of range management, 41*, 282–286.

Heitschmidt, R. K., Dowhower, S. L., & Walker, J. W. (1987). Some effects of a rotational grazing treatment on quantity and quality of available forage and amount of ground litter. *Journal of Range Management, 40*, 318-321.

Hendershot, K. L., & Volenec, J. J. (1993). Taproot nitrogen accumulation and use in overwintering alfalfa (*Medicago sativa* L.). *Journal of plant physiology, 141*(1), 68-74.

Hendricksen, R., & Minson, D. J. (1980). The feed intake and grazing behaviour of cattle grazing a crop of *Lablab purpureus* cv. Rongai. *The Journal of Agricultural Science*, *95*(03), 547-554.

Hill laboratories, South Island (2013). Pasture and feed forage quality. *Technical paper 3.* Retrieved 22/08, 2013, from http://www.hill-laboratories.com/page/pageid/2145845337/Technical_notes

Hodgson, J. (1981). Variations in the surface characteristics of the sward and the short-term rate of herbage intake by calves and lambs. *Grass and Forage Science, 36,* 49–57.

Hodgson, J., & Brookes, I. M. (1999). Nutrition of grazing animals. In 'New Zealand pasture and crop science'.(Eds J.G.H., White, J. Hodgson) pp. 117–132: Oxford University Press: Melbourne.

Hof, G., Vervoorn, M. D., Lenaers, P. J., & Tamminga, S. (1997). Milk urea nitrogen as a tool to monitor the protein nutrition of dairy cows. *Journal of dairy science, 80*(12), 3333-3340.

Hoglund, J. H., Dougherty, C. T., & Langer, R. H. (1974). Response of irrigated lucerne to defoliation and nitrogen fertiliser. *New Zealand of Experimental Agriculture 2*, 7-11.

Holden, L. A., Muller, L. D., Lykos, T., & Cassidy, T. W. (1995). Effect of corn silage supplementation on intake and milk production in cows grazing grass pasture. *Journal of dairy science, 78*(1), 154-160.

Holmes, C. W., & Matthews, P. N. P. (2001). Feeding of conserved forage-implications to grassland management and production *Proceedings of the XIX International Grassland Congress* (pp. 671-677). Piracicaba, Brazil, FEALQ.

Holmes, C. W., Wilson, G. F., Mackenzie, D. D. S., Flux, D. S., Brookes, I. M., & Davey, A. W. F. (2002). *Milk production from pasture.* (3rd ed.). Wellington: Butterworths of New Zealand.

Homes, C. W., & Roche, J. R. (2007). Pastures and Supplements in New Zealand Dairy Production Systems. In P.V. Rattray (Ed.), *Pastures and supplements for grazing animals. Occasional Publication, No.14.* . Hamilton: New Zealand Society of Animal Production.

Howarth, R.E., Chaplin, R.K., Cheng, K.J., Goplen, B.P., Hall, J.W., Hironaka, R., . . . Radostits, O.M. (1991). *Bloat in cattle*. Ottawa, Ontario, Canada,: Communications Branch, Agriculture Canada.

Huhtanen, Pekka, & Hristov, A. N. (2009). A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. *Journal of dairy science, 92*(7), 3222-3232.

INRA, (Institut National de la Recherche Agronomique). (2007). *Alimentation des bovins, ovins et caprins, Quae Eds*. Versailles, France.

Iversen, C. E. (1967). Grazing manage.ment of lucerne. In R. H. M. Langer (Ed.), *The Lucerne Crop* (pp. 74-84). Reed, Wellington.

Jagusch, K.T. (1982). Nutrition of ruminants grazing lucerne. In R.B. Wynn-Williams (Ed.), *Lucerne for the 80's. Agronomy Society of New Zealand special publication No.1* (pp. 73-78).

Jarvis, S. C., Wilkins, R. J., & Pain, B. F. (1996). Opportunities for reducing the environmental impact of dairy farming managements: a systems approach. *Grass and Forage Science, 51*(1), 21-31.

Jarvis, SC, Hatch, DJ, & Roberts, Daphne H. (1989). The effects of grassland management on nitrogen losses from grazed swards through ammonia volatilization; the relationship to excretal N returns from cattle. *Journal of Agricultural Science*, *112*, 205-216.

Jonker, J. S., Kohn, R. A., & Erdman, R. A. (1998). Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. *Journal of dairy science, 81*(10), 2681-2692.

Journet, M., & Demarquilly, C. (1979). Grazing. In W. H. Broster & H. Swan (Eds.), *Feeding strategy for the high yielding cow, EAAP publication no. 25* (pp. 295-321). London, : Granada Publishing.

Keady, T.W.J., Mayne, C.S., & Marsden, M. (1998). The effects of concentrate energy source on silage intake and animal performance with lactating dairy cows offered a range of grass silages. *Animal Science, Glasgow, 66*, 21-34.

Kebreab, E., France, J., Beever, D. E., & Castillo, A. R. (2001). Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. *Nutrient Cycling in Agroecosystems, 60*(1), 275-285.

Kellaway, R. C., & Porta, S. (1993). Factors affecting the response to supplementation. In R. Hopkins (Ed.), *Feeding concentrates: supplements for dairy cows* (pp. 117-147). Melbourne.: Agmedia,.

Kellaway, R. C., Tassell, R. J., Havilah, E., Sriskandarajah, N., & Andrews, A. (1993). Nutrient balance in the diet of grazing dairy cows. *Crop and Pasture Science, 44*(3), 423-430.

Kenny, D.A., Boland, M.P., Diskin, M.G., & Sreenan, J.M. (2001). Effect of pasture crude protein and fermentable energy supplementation on blood metabolite and progesterone concentrations and on embryo survival in heifers. *Animal Science, 73*, 501-511.

Kenny, D.A., Boland, M.P., Diskin, M.G., & Sreenan, J.M. (2002). Effect of rumen degradable protein with or without fermentable carbohydrate supplementation on blood metabolites and embryo survival in cattle. *Animal Science, 74*, 529-537.

Keoghan, J. M. (1991). *Future Options for Dryland Pastures*. Paper presented at the Supporting Papers for the New Zealand Meat Research and Development

Council-sponsored Field Day, Belfield, Hakataramea, New Zealand. http://books.google.co.nz/books?id=sYcYMgAACAAJ

Khaiti, M., & Lemaire, G. (1992). Dynamics of shoot and root growth of lucerne after seeding and after cutting [radiation use efficiency, shoot/root ratio, seasonal effect]. *European Journal of Agronomy, 1*.

Kibon, A., & Holmes, W. (1987). The effect of height of pasture and concentrate composition on dairy cows grazed on continuously stocked pastures. *The Journal of Agricultural Science, 109*(02), 293-301.

Kim, T. H., Ourry, A., Boucaud, J., & Lemaire, G. (1993). Partitioning of nitrogen derived from N2 fixation and reserves in nodulated *Medicago sativa* L. during regrowth. *Journal of experimental Botany, 44*(3), 555-562.

Kirkland, R. M., & Gordon, F. J. (2001). The effects of stage of lactation on the partitioning of, and responses to changes in, metabolisable energy intake in lactating dairy cows. *Livestock Production Science, 72*(3), 213-224.

Kirsopp, S. (2001). *Management techniques to maximise legume production in dryland farming* . (Masters of Applied Science (MSc)), Lincoln University, Christchurch.

Kleinig, C. R., Noble, J. C., & Rixon, A. J. (1974). Herbage production and the accumulation of soil nitrogen under irrigated pastures on the Riverine Plain. *Animal Production Science, 14*(66), 49-56.

Kleinschmidt, F. H. (1967). The influence of nitrogen and water on pastures of green panic, lucerne, and glycine at Lawes, South-eastern Queensland. *Animal Production Science, 7*(28), 441-446.

Knight, T.L. (1987). *Sheep production on dryland lucerne*. Paper presented at the Proceedings of the New Zealand Grassland Association.

Kohn, R. A., Dinneen, M. M., & Russek-Cohen, E. (2005). Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. *Journal of Animal Science, 83*(4), 879-889.

Kolver, E., Muller, L. D., Varga, G. A., & Cassidy, T. J. (1998a). Synchronization of Ruminal Degradation of Supplemental Carbohydrate with Pasture Nitrogen in Lactating Dairy Cows. *Journal of dairy science, 81*(7), 2017-2028.

Kolver, E. S., Muller, L. D., Barry, M. C., & Penno, J. W. (1998b). Evaluation and Application of the Cornell Net Carbohydrate and Protein System for Dairy Cows Fed Diets Based on Pasture. *Journal of Dairy Science, 81*(7), 2029-2039. doi: http://dx.doi.org/10.3168/jds.S0022-0302(98)75777-7

Kolver, Eric. (2000). *Nutrition guidelines for the high producing dairy cow*. Paper presented at the Ruakura farmers conference.

L'Huillier, P. J., Poppi, D. P., & Fraser, T. J. (1984). Influence of green leaf distribution on diet selection by sheep and the implications for animal performance. *Proceedings of the New Zealand Society of Animal Production, 44*, 105-107.

Langer, R. H. M. (1973). Pastures and pasture plants (pp. 428). Auckland: Oxford University Press.

Lantinga, E., & Groot, J. (1996). Optimization of grassland production and herbage feed quality in an ecological context *Utilization of Local Feed Resources by Dairy Cattle. Proceedings of an International Symposium,* (Vol. 84, pp. 58–66). Wageningen, The Netherlands.: EAAP Publication.

Larson, S. F., Butler, W. R., & Currie, W. B. (1997). Reduced Fertility Associated with Low Progesterone Postbreeding and Increased Milk Urea Nitrogen in Lactating Cows. *Journal of Dairy Science, 80*(7), 1288-1295. doi: http://dx.doi.org/10.3168/jds.S0022-0302(97)76058-2

Leaver, J. D. (1985). Milk production from grazed temperate grassland. *Journal of Dairy Research, 52*(2), 313-344.

Leaver, J. D., Campling, R. C., & Holmes, W. (1968). Use of supplementary feeds for grazing dairy cows. *Dairy Science Abstracts, 30*(7), 355-361.

Ledgard, S. F. (2006). Nitrogen management–why is it important and what can we do about it? *Proceedings of the 2006 Dairy3 Conference 4* (pp. 23-31).

Ledgard, S. F., de Klein, C.A.M., Crush, J.R., & Thorrold, B.S. (2000). Dairy farming, nitrogen losses and nitrate-sensitive areas *New Zealand Society of Animal Production, 60*, 256-260.

Limon Sanchez, O., & Campling, R. C. (1982). Comparison of milk production from cows grazing lucerne and ryegrass pastures. . *Grass and Forage Science, 37*, 172-173.

Lodge, G.M. (1991). Management practices and other factors contributing to the decline in persistence of grazed lucerne in temperate Australia: a review. *Australian Journal of Experimental Agriculture, 31,* 813-724.

Macdonald, K. (1999). Determining How To Make Inputs Increase Your Economic Farm Surplus *Proceedings of the Ruakura dairy Farmers' Conference 51.* (pp. 78-87). Hamilton, New Zealand: Dairy Research Corporation.

Mace, M. J. (1982). Grazing management in practice – North Island Dairying. In R. B. Wynn Williams (Ed.), *Lucerne for the 80s Special Publication No.1*. Palmerston North, New Zealand: Agronomy Society of New Zealand

Majak, W., Hall, J.W., & McCaughey, W.P. (1995). Pasture management strategies for reducing the risk of legume bloat in cattle. *Journal of animal science, 73*(5), 1493-1498.

Majak, W., McAllister, T.A., McCartney, D., Stanford, K., & Cheng, K.J. (2003). *Bloat in cattle* (C. Kaulbars Ed.). Edmonton: Alberta Agriculture, Food and Rural Development, Information Packaging Centre.

Mayne, C. S. (1991). Effects of supplementation on the performance of both growing and lactating cattle at pasture. *Management issues for the grassland farmer in the 1990's*, 55-71.

McCoy, J. E, Collins, M, & Dougherty, C.T. (1997). Amount and quality of chicory herbage ingested by grazing cattle. *Crop Science, 37*, 239–242. doi: https://www-crops-org.ezproxy.lincoln.ac.nz/publications/cs

McEvoy, M., Kennedy, E., Murphy, J. P., Boland, T. M., Delaby, L., & O’donovan, M. (2008). The effect of herbage allowance and concentrate supplementation on milk production performance and dry matter intake of spring-calving dairy cows in early lactation. *Journal of dairy science, 91*(3), 1258-1269.

McGilloway, D. A., Cushnahan, A., Laidlaw, A. S., Mayne, C. S., & Kilpatrick, D. J. (1999). The relationship between level of sward height reduction in a rotationally grazed sward and short-term intake rates of dairy cows. *Grass and Forage Science, 54*(2), 116-126.

McGilloway, D. A., & Mayne, C. S. (1996). The importance of grass availability for the high genetic merit dairy cow. In P. C. Garnsworthy, J. Wiseman & W. Haresign (Eds.), *Recent advances in animal nutrition.* (pp. 135). UK: Nottingham University Press.

McGowan, A.W., Sheath, G.W., Webby, R.W., & Moot, D.J. (2003). *Lucerne for high quality summer feed in North Island hill country.* Paper presented at the Legumes for dryland pastures. Proceedings of a New Zealand Grassland Association (Inc.) Symposium held at Lincoln University, 18-19 November, 2003.

Meijs, J. A. C., & Hoekstra, J. A. (1984). Concentrate supplementation of grazing dairy cows. 1. Effect of concentrate intake and herbage allowance on herbage intake. *Grass and forage science., 39*(1), 59–66.

Michaud, R., Lehman, W. F., & Rumbaugh, M. D. (1988). World Distribution and Historical Development. In A. A. Hanson, D. K. Barnes & R. R. Hill Jr. (Eds.), *Alfalfa and Alfalfa improvement* (Vol. 29). Madison, Wisconsin: American Society of Agronomy,Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc,.

Miller, L.A., Moorby, Jon M., Davies, David R., Humphreys, Mervyn O., Scollan, Nigel D., MacRae, John C., & Theodorou, Michael 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*(4), 383-394.

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

Mills, A., Smith, M. C., Lucas, R. J. , & Moot, D. J. (2008). Dryland pasture yields and botanical composition over 5 years under sheep grazing in Canterbury. *Proceedings of the New Zealand Grassland Association*, *70,* 37-44.

Milne, G. D. (2011). Can pasture persistence be improved through the use of non-ryegrass species? In C. F. Mercer (Ed.), *Pasture Persistence Symposium. Grassland Research and Practice Series No. 15* (pp. 157-162). Dunedin: New Zealand Grassland Association.

Minson, D. J. (1971). The digestibility and voluntary intake of six varieties of *Panicum*. *Animal Production Science, 11*(48), 18-25.

Minson, Dennis. (1990). *Forage in ruminant nutrition*: Access Online via Elsevier.

Moate, P. J., Dalley, D. E., Roche, J. R., & Grainger, C. (1999). Dry matter intake, nutrient selection and milk production of dairy cows grazing rainfed perennial pastures at different herbage allowances in spring. *Australian Journal of Experimental Agriculture, 39*(8), 923-931. doi: http://dx.doi.org/10.1071/EA99022

Moorby, Jon M., Evans, Roger T., Scollan, Nigel D., MacRae, John C., & Theodorou, Michael 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*(1), 52-59.

Moot, D., Avery, D., & Avery, F. (2009). Seasonal priorities for successful grazing management of lucerne. Starborough, New Zealand: Flaxbourne Soil Conservation Project.

Moot, D. J. (2009). Review of Lucerne in New Zealand *New Zealand Farm Environment Trust and Ministry of Agriculture and Fisheries*.

Moot, D. J., Brown, H. E., Teixeira, E. I., & Pollock, K. M. (2003). Crop growth and developement affect seasonal priorities for lucerne management. *In:* D.J. Moot (ed). Legumes for dryland pastures. *Proceeding of the New Zealand Grasslands association*.

Morrison, S. J., & Patterson, D. C. (2007). The effects of offering a range of forage and concentrate supplements on milk production and dry matter intake of grazing dairy cows. *Grass and Forage Science, 62*(3), 332-345.

Nocek, J.E., & Russell, J.B. (1988). Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *Journal of Dairy Science, 71*(8), 2070-2107.

NRC, National Research Council. (1989). *Nutrient requirements of dairy cows. Sixth revised edition* (Sixth ed.). Washington, DC.: National Academy of Science,.

Ordóñez, A., Matthew, C., Miller, R.D., Parkinson, T.J., Holmes, CW, Brookes, I.M., . . . Brookes, I. M. (2004). *Does spring application of fertiliser urea reduce dairy cow performance.* Paper presented at the Proceedings of the New Zealand Grassland Association.

Ordonez, A., Parkinson, T.J., Matthew, C., Holmes, C.W., Miller, R.D., Lopez-Villalobos, N., . . . Brookes, I. M. (2007). Effects of application in spring of urea fertiliser on aspects of reproductive performance of pasture-fed dairy cows. *New Zealand veterinary journal, 55*(2), 69-76.

Orr, R.J., Rutter, S.M., Penning, P.D., & Rook, A.J. (2001). Matching grass supply to grazing patterns for dairy cows. *Grass and Forage Science, 56*(4), 352-361.

Pacheco, D. , Lowe, K. , Burke, J. L. , & Cosgrove, G. P. (2009). *Urinary nitrogen excretion from cows at different stage of lactation grazing different ryegrass cultivars during spring or autumn* (Vol. 69): New Zealand Society of Animal Production.

Pacheco, D., Burke, J. L., & Cosgrove, G. P. (2007). *An empirical model to estimate efficiency of nitrogen utilisation in cows grazing fresh forages.* Paper presented at the Meeting the challenges for pasture-based dairying. Proceedings of the 3rd Dairy Science Symposium. Eds. Chapman, DF.

Pecetti, L., Romani, M., & Piano, E. (2006). Persistence of morphologically diverse lucerne under continuous stocking and intensive grazing. *Crop and Pasture Science, 57*(9), 999-1007.

Pehrson, B, Forshell, K, & Carlsson, J. (1992). The Effect of Additional Feeding on the Fertility of High-yielding Dairy Cows. *Journal of Veterinary Medicine series A, 39*(1-10), 187-192.

Penno, J. W. (2002). *The response by grazing dairy cows to supplementary feeds.* (Ph. D. Thesis), Massey University, New Zealand.

Penno, J. W., Holmes, C. W., MacDonald, K. A., & Walsh, B. J. (1998). *The effect of stage of lactation and season on milksolids response to supplementary feeding of dairy cows.* Paper presented at the Proceedings of New Zealand Society of Animal Production.

Penno, J. W., Macdonald, K. A., & Holmes, C. W. (2001). Toward a predictive model of supplementary feeding response from grazing dairy cows. *Proceedings of New Zealand Society of Animal Production, 61*, 229-233.

Penno, J. W., Macdonald, K. A., Holmes, C. W., Davis, S. R., Wilson, G. F., Brookes, I. M., & Thom, E. R. (2006). Responses to supplementation by dairy cows given low pasture allowances in different seasons 1. Pasture intake and substitution. *Animal Science, 82*(05), 661-670.

Peyraud, J. L., Vérité, R., & Delaby, L. (1995). Rejets azotés chez la vache laitière: effets du type d'alimentation et du niveau de production des animaux. *Fourrages, 142*, 131-144.

Peyraud, J.L., & Astigarraga, L. (1998). Review of the effect of nitrogen fertilization on the chemical composition, intake, digestion and nutritive value of fresh herbage: consequences on animal nutrition and N balance. *Animal Feed Science and Technology, 72*(3), 235-259.

Peyraud, J.L., Astigarraga, L., & Faverdin, P. (1997). Digestion of fresh perennial ryegrass fertilized at two levels of nitrogen by lactating dairy cows. *Animal Feed Science and Technology, 64*(2), 155-171.

Phillips, C. J. C. (1993). Nutritional behaviour. In Phillips C. J. C (Ed.), *Cattle Behaviour* (pp. 75). UK: Farming Press.

Phyn, C. V. C. , Clark, C. , Aspin, P. W. , & Kolver, E. S. (2008). *Effect of genotype and diet on feed conversion efficiency of dairy cows during a 600-day extended lactation* (Vol. 68): New Zealand Society of Animal Production.

Pinchak, W.E., Min, B.R., Malinowski, D.P., Sij, J.W., Gill, R.J., Puchala, R., & Anderson, R.A. (2005). Re-evaluation of the frothy bloat complex in cattle grazing winter wheat in the southern plains. Evolution of a new integrated paradigm. *Proceedings of the 2005 Conference on Gastrointestinal Function, 11th–13th April, Chicago, Illinois, USA*, 36.

Pioneer. (2010). Lucerne manual. In Pioneer® brand products (Ed.), *Pioneer Hi-Bred International, Inc.* (pp. 31-33). New Zealand: Genetic Technologies Limited.

Popp, J.D., McCaughey, W.P., & Cohen, R.D.H. (1997). Effect of grazing system, stocking rate and season of use on herbage intake and grazing behaviour of stocker cattle grazing alfalfa-grass pastures. *Canadian Journal of Animal Science, 77*(4), 677-682.

Popp, J.D., McCaughey, W.P., Thomas, L.R., & Cohen, R.D.H. (1999). Nutrient selection by cattle from grass and grass/legume pastures. *Canadian journal of animal science, 79*(3), 391-395.

Poppi, D. P., & McLennan, S. R. (1995). Protein and energy utilization by ruminants at pasture. *Journal of Animal Science, 73*(1), 278-290.

Poppi, D. P., Minson, D. J., & Ternouth, J. H. (1981). Studies of cattle and sheep eating leaf and stem fractions of grasses, 1. The voluntary intake, digestibility and retention time in the reticulo-rumen. *Australian Journal of Agricultural Research, 32*, 123–137.

Pulido, R. G., & Leaver, J. D. (2001). Quantifying the influence of sward height, concentrate level and initial milk yield on the milk production and grazing behaviour of continuously stocked dairy cows. *Grass and Forage Science, 56*(1), 57-67.

Rajala-Schultz, P. J., & Saville, W. J. A. (2003). Sources of variation in milk urea nitrogen in Ohio dairy herds. *Journal of dairy science, 86*(5), 1653-1661.

Rajala-Schultz, P. J., Saville, W. J. A., Frazer, G. S., & Wittum, T. E. (2001). Association Between Milk Urea Nitrogen and Fertility in Ohio Dairy Cows. *Journal of Dairy Science, 84*(2), 482-489. doi: http://dx.doi.org/10.3168/jds.S0022-0302(01)74498-0

Ralphs, M. H., Kothmann, M. M., & Merrill, L. B. (1986). Cattle and sheep diets under short-duration grazing. *Journal of Range Management, 39*, 217-223.

Rattray, P. V., Brookes, I. M., & Nicol, A. M. (2007). Pasture and supplements for grazing animals. In P.V. Rattray (Ed.), *Pasture and Supplements for Grazing Animals. Occasional Publication No. 14*, (pp. 221-239). Hamilton: New Zealand Society of Animal Production.

Richardson, A. E. V., & Gallus, H. P. C. (1932). Investigations on Irrigated Pastures: 1. The Yield and Botanical Composition of an Irrigated Permanent Pasture Under Various Systems of Pasture Management 2. The chemical composition of irrigated pastures at Wood's point. (Vol. 71, pp. 45). South Australia.: C.S.I.R. Bull.

Roche, J. R. (Producer). (2010). Too much protein in spring does not reduce pregnancy rate? *Inside Dairy: Myth Busters*. Retrieved from http://www.dairynz.co.nz/page/pageid/2145881027/Myth_Busters

Roche, J.R., Petch, S., & Kay, J.K. (2005). Manipulating the dietary cation-anion difference via drenching to early-lactation dairy cows grazing pasture. *Journal of dairy science, 88*(1), 264-276.

Rook, A. J. (2000). Principles of foraging and grazing behaviour. In 'Grass: its production and utilization'.(Ed. A. Hopkins) pp. 229–246: Blackwell Science Ltd: Malden, MA.

Rook, A. J., Huckle, C. A., & Penning, P. D. (1994). Effects of sward height and concentrate supplementation on the ingestive behaviour of spring-calving dairy cows grazing grass-clover swards. *Applied Animal Behaviour Science, 40*(2), 101-112.

Rutter, S. M., Champion, R. A., & Penning, P. D. (1997). An automatic system to record foraging behaviour in free-ranging ruminants. *Applied Animal Behaviour Science, 54*(2), 185-195.

Sairanen, Auvo, Khalili, Hannele, Nousiainen, Jouni I, Ahvenjärvi, Seppo, & Huhtanen, Pekka. (2005). The effect of concentrate supplementation on nutrient flow to the omasum in dairy cows receiving freshly cut grass. *Journal of dairy science, 88*(4), 1443-1453.

Sayers, H. J. (1999). *The effect of sward characteristics and level and type of supplement on grazing behaviour, herbage intake and performance of lactating dairy cows.* (Ph.D Thesis), Queen's University of Belfast, Hillsborough.

Schlegel, M. L., Wachenheim, C. J., Benson, M. E., Ames, N. K., & Rust, S. R. (2000). Grazing methods and stocking rates for direct-seeded alfalfa pastures: II. Pasture quality and diet selection. *Journal of animal science, 78*(8), 2202-2208.

Sewell, J.C., Hill, R.D., & Reich, J. (2011). Persistence of grazing tolerant lucernes under Australian conditions. In C.F. Mercer (Ed.), *Pasture Persistence Symposium. Grassland Research and Practice Series No. 15* (pp. 181-186). Dunedin: New Zealand Grassland Association.

Sheahan, A. J., Kolver, E. S., & Roche, J. R. (2011). Genetic strain and diet effects on grazing behavior, pasture intake, and milk production. *Journal of Dairy Science, 94*(7), 3583-3591. doi: http://dx.doi.org/10.3168/jds.2010-4089

Shingfield, K. J., & Offer, N. W. (1998). Evaluation of the spot urine sampling technique to assess urinary purine derivative excretion in lactating dairy cows. *Animal Science, 66*(03), 557-568. doi: doi:10.1017/S1357729800009139

SIDDC. (2010). South Island Dairying Development Centre - Pasture analysis (LUDF). Retrieved 08th December,, 2012, from http://www.siddc.org.nz/lincolnuniversitydairyfarm/ludfpastureanalysis.html

Simpson, J. R. (1976). Transfer of nitrogen from three pasture legumes under periodic defoliation in a field environment. *Animal Production Science, 16*(83), 863-870.

Smith, G. , Bryant, R. H. , & Edwards, G. R. (2013). *Milksolids production of dairy cows grazing lucerne and perennial ryegrass in spring* (Vol. 73): New Zealand Society of Animal Production.

Smith, J.F., Beaumont, S., Hagemann, L., McDonald, R.M., Peterson, A.J., Xu, Z.Z., & Duganzich, D.M. (2001). *Relationship between bulk milk urea nitrogen and reproductive performance of New Zealand dairy herds.* Paper presented at the Proceedings of New Zealand Society of Animal Production.

Smith, K.A., & Frost, J.P. (2000). Nitrogen excretion by farm livestock with respect to land spreading requirements and controlling nitrogen losses to ground and surface waters. Part 1: cattle and sheep. *Bioresource Technology, 71*(2), 173-181.

Stobbs, T.H. (1974). Rate of biting by Jersey cows as influenced by the yield and maturity of pasture swards. *Tropical Grassld, 8*, 81-86.

Stockdale, C. R. (1997). Supplements improve the production of dairy cows grazing either white clover or paspalum-dominant pastures in late lactation. *Australian journal of experimental agriculture., 37*(3), 295-302.

Stockdale, C. R. (2000). Differences in body condition and body size affect the responses of grazing dairy cows to high-energy supplements in early lactation. *Animal Production Science, 40*(7), 903-911.

Stockdale, C. R., Callaghan, A., & Trigg, T. E. (1987). Feeding high energy supplements to pasture-fed dairy cows. Effects of stage of lactation and level of supplement. *Crop and Pasture Science, 38*(5), 927-940.

Stockdale, C. R., & Trigg, T. E. (1989). Effect of pasture feeding levels on the responses of lactating dairy cows to high energy supplements. *Australian Journal of Experimental Agriculture, 29*(5), 605-611. doi: http://dx.doi.org/10.1071/EA9890605

Talke, H., & Schubert, G. E. (1965). Enzymatische Harnstoffbestimmung in Blut und Serum im optischen Test nachWarburg. *Journal of Molecular Medicine, 43*(3), 174-175. doi: 10.1007/bf01484513

Tamminga, Seerp, Bannink, A, Dijkstra, J, & Zom, R. (2007). *Feeding strategies to reduce methane loss in cattle*.

Tas, B. M., & Susenbeth, A. (2007). Urinary purine derivates excretion as an indicator of in vivo microbial N flow in cattle: A review. *Livestock Science, 111*(3), 181-192. doi: http://dx.doi.org/10.1016/j.livsci.2007.05.010

Teakle, J. H. (1957). Lucerne investigations on the biloela regional experimental station. *Queensland Agricultural Journal, 83*, 239-247.

Teixeira, Edmar I., Moot, Derrick J., Brown, Hamish E., & Fletcher, Andrew L. (2007). The dynamics of lucerne (*Medicago sativa* L.) yield components in response to defoliation frequency. *European journal of agronomy, 26*(4), 394-400.

Thom, E.R., & Smith, G.S. (1980). Effect of stage of growth and season on the major element composition of the aerial parts of lucerne (Medicago sativa L.). *Proceedings of Agronomy Society of New Zealand, 10*, 23-26.

Thompson, D.J., Brooke, B.M., Garland, G.J., Hall, J.W., & Majak, W. (2000). Effect of stage of growth of alfalfa on the incidence of bloat in cattle. *Canadian Journal of Animal Science, 80*(4), 725-727.

Thomson, N. A., & Lagan, J. F. (1981). Lucerne not the answer in dairying. *New Zealand Journal of Agriculture (May 1991), 13*, 17.

Totty, V. K, Greenwood, S. L., Bryant, R. H, & Edwards, G. R. (2012). Nitrogen partitioning and milk production of dairy cows grazing simple and diverse pastures. *Journal of dairy science*.

Trevaskis, L. M., Fulkerson, W. J., & Nandra, K. S. (2004). Effect of time of feeding carbohydrate supplements and pasture on production of dairy cows. *Livestock Production Science, 85*(2), 275-285.

Tyrrell, H. F., Moe, P. W., & Flatt, W. P. (1970). Influence of excess protein intake on energy metabolism of the dairy cow. *Energy Metabolism of Farm Animals. EAAP Publ*(13), 69-72.

Ulyatt, M. J., Lancashire, J. A., & Jones, W. T. (1977). *The nutritive value of legumes.* Paper presented at the Proceedings of the New Zealand Grassland Association.

Valk, H. (1994). Effect of partial replacement of herbage by maize silage on N-utilisation and milk production of dairy cows. *Livestock production science, 40*, 241-250.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of dairy science, 74*(10), 3583-3597.

Van Vuuren, A. M., & Mejis, J. A. C. (Eds.). (1987). *Effects of herbage composition and supplement feeding on the excretion of nitrogen in dung and urine by grazing dairy cows*: Dordrecht.

Van Vuuren, A.M., Krol-Kramer, F., Van der Lee, R.A., & Corbijn, H. (1992). Protein Digestion and Intestinal Amino Acids in Dairy Cows Fed Fresh *Lolium perenne* with Different Nitrogen Contents. *Journal of dairy science, 75*(8), 2215-2225.

Wade, M. H., Peyraud, J. L., Lemaire, G., & Comeron, E. A. (1989). The dynamics of daily area and depth of grazing and herbage intake of cows in a five day paddock system *Proceedings of the 16th International Grassland Congress* (pp. 1111-1112). Nice, France.

Waghorn, G.C., Burke, J. L., & Kolver, E. S. (2007). Principles of feeding value. In P. Rattray, I.M. Brookes & A. Nicol (Eds.), *Pasture and Supplements for Grazing Animals.* (Vol. Occasional Publication 14, pp. 35-59). Hamilton, New Zealand: New Zealand Society of Animal Production.

Wales, W. J., Doyle, P. T., & Dellow, D. W. (1998). Dry matter intake and nutrient selection by lactating cows grazing irrigated pastures at different pasture allowances in summer and autumn. *Australian Journal of Experimental Agriculture, 38*(5), 451-460. doi: http://dx.doi.org/10.1071/EA98043

Wales, W. J., Stockdale, C. R., Doyle, P. T., & Dellow, D. W. (1999). Effects of variations in herbage mass, allowance, and level of supplement on nutrient intake and milk production of dairy cows in spring and summer. *Animal Production Science, 39*(2), 119-130.

Wales, W. J., Williams, Y. J., & Doyle, P. T. (2001). Effect of grain supplementation and the provision of chemical or physical fibre on marginal milk production responses of cows grazing perennial ryegrass pastures. *Australian Journal of Experimental Agriculture, 41*(4), 465-471. doi: http://dx.doi.org/10.1071/EA00156

Walker, J. W., Heitschmidt, R. K., De Moraes, E. A., Kothmann, M. M., & Dowhower, S. L. (1989). Quality and botanical composition of cattle diets under rotational and continuous grazing treatments. *Journal of Range Management*, 239-242.

Walton, P. D., Martinez, R., & Bailey, A. W. (1981). A comparison of continuous and rotational grazing. *Journal of Range Management*, 19-21.

White, J. G. H. (1982). Lucerne grazing management for the 80’s. In Wynn-Williams (Ed.), *Lucerne for the 80's, Special Publication 1* (Vol. 1, pp. 111 - 116). Palmerston North, New Zealand: Agronomy Sociecty of New Zealand (Inc.).

White, J.G.H., & Cosgrove, G.P. (1990). Lucerne grazing management 2. Effect of grazing duration on defoliation pattern by ewes. *New Zealand Journal of Agricultural Research, 33*(4), 621-625.

Wildman, E.E., Jones, G.M., Wagner, P.E., Bowman, R.L., Troutt, H.F., & Lesch, T.N. (1982). A dairy cow body condition scoring system and its relationship to selected production characteristics. *ournal of Dairy Science, 65*, 495–501.

Wills, G.A., & Holmes, C.W. (1988). *Effect of supplementary hay fed to non-lactating cows offered two allowances of pasture.* Paper presented at the Proceedings of the New Zealand Grassland Association.

Woodward, S. L., Auldist, M. J. , Laboyrie, P. J. , & Jansen, E. B. L. (1999). Effect of *Lotus corniculatus* and condensed tannins on milk yield and composition of dairy cows. *Proceedings of the New Zealand Society of Animal Production, 59*, 152-155.

Woodward, S. L., Chaves, A. V., Waghorn, G. C., & Laboyrie, P. J. (2002a). *Supplementing pasture-fed dairy cows with pasture silage, maize silage, Lotus silage or sulla silage in summer-does it increase production?* Paper presented at the Proceedings of the conference-New Zealand grassland association.

Woodward, S. L., Roach, C. G., MacDonald, K. A., & Siemlink, J. C. (2008). Forage mixed ration farming-the pros and cons. *Proceeding of the New Zealand Grasslands association, 70*, 183-188.

Woodward, S. L., Waghorn, G. C., Attwood, G. C., & Li, D. (2010). Ryegrass to lucerne-effects of dietary change on intake, milk yield and rumen microflora bacteria of dairy cows. *Proceedings of the New Zealand Society of Animal Production, 70*, 57-61.

Woodward, S. L., Waghorn, G. C., Lassey, K. R., & Laboyrie, P. G. (2002b). Does feeding sulla (*Hedysarum coronarium*) reduce methane emissions from dairy cows? *Proceedings of New Zealand Society of Animal Production*, *62*, 227-230.

Woodward, Sharon, Chaves, Alex, Grayling, Liz, & Waghorn, Garry. (2001). *Specialist Silages For Increased Milksolids Production In Late Summer-Autumn.* Paper presented at the Ruakura Farmers Conference.

Wu, Z., & Satter, L.D. (2000). Milk production during the complete lactation of dairy cows fed diets containing different amounts of protein. *Journal of Dairy Science, 83*(5), 1042-1051.