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**Effect of energy and rumen undegradable protein
supplementation on milk production and nitrogen losses in dairy
cows**

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

at
Lincoln University
by
Sophie Remail Parker

Lincoln University
2012

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The rising milk production potential of the NZ dairy herd is reducing the ability for farmers to meet cow energy demands. Providing a higher proportion of the CP in the diet as RUP has been identified as a possible way to increase the protein supply to the animal. Alternately, modulating the nutrient dynamics in the rumen has the potential to capture more of the excess CP available from pasture.

The objectives of this research were to measure the milk solid, urinary and faecal nitrogen responses of dairy cows fed iso-energetic diets supplemented with wheat and canola meal, compared to pasture-only diets and to measure these same variables when the timing of supplementation, relative to pasture allocation was altered (1 h or 9 h post-pasture).

It was hypothesised that the supplemented treatments would have increased milk production and decreased urinary and faecal N losses, compared to the control. It was predicted that the supplemented treatments would not differ significantly from each other in milk production, but that both supplemented groups would have lower urinary and faecal N losses. It was also hypothesised that the 9 h treatments would have significantly higher milk production and lower urinary and faecal N losses than the 1 h treatments.

A total of 30 Friesian x Jersey cows (liveweight: 455 kg) were all matched for parity, body condition score (BCS) and days in milk (DIM), and allocated to five treatment groups (six cows/ treatment). The cows underwent a 7 d adjustment period before undergoing a 24 d treatment period. Four of the treatment groups received either 5.1 kg DM rolled wheat grain or wheat and canola meal, as well as 11.5 kg DM pasture. The treatment groups included: C: pasture (p); E9: p + wheat (w) 9 hours (h) before p; E1: p + w 1 h before p; P9: p + w and

canola meal (c) 9 h before p; P1: p + w and c 1 h before p. All of the supplemented treatment groups were designed to be iso-energetic; with the high-RUP supplemented group being iso-nitrogenous with the pasture-only treatment group. Measurements included: milk yield, milk composition, faecal, urine, blood, pasture and concentrate samples. The data were analysed in a two-step analysis that first included the control group in an ANOVA model in GenStat and then the four supplemented groups were analysed using a 2 x 2 unbalanced factorial ANOVA model.

Providing a RFCHO source above a pasture-only diet significantly increased MS production in one of the wheat treatments (2.2 kg/c/d, compared to 2.0 kg/c/d in the control), but not in the other (1.9 kg/c/d) ($P < 0.001$). Nitrogen losses in the wheat-supplemented treatments were lower than in the C group. When the N percentage in the urine and faeces of the E groups was compared with the C group, it was shown that urinary N was reduced by 12.1% ($P = 0.038$) and faecal N by 7.4% ($P < 0.001$).

Feeding a high-RUP diet to cows (compared to an iso-energetic low-RUP diet) increased the level of MS production by 0.2 kg/d and 0.1 kg/d in the P9 and P1 groups, respectively ($P < 0.001$). The P groups lost an average 0.33 of a body condition score (BCS) during the experiment, compared to a 0.07 BCS loss in the E groups ($P = \text{NS}$). The P groups urinary and faecal N losses (as a percentage of N intakes) were higher than the E groups (2.6% and 1.8% increases in urinary and faecal losses, respectively).

Timing supplementation to occur 9 h pre-pasture intake was seen to decrease MS production, compared to offering the supplement 1 h pre-pasture allocation (2.04 MS/d, compared to 2.15 MS/d) ($P = \text{NS}$). The 9 h treatment groups increased in LWT over the experimental period by 18.2 kg; compared to 6.3 kg in the 1 h groups ($P = \text{NS}$). There was a non-significant 4.1 % and 3.2 % reduction in urinary and faecal N losses in the 9 h group, compared to the 1 h group.

The implications of the trial are that feeding a concentrate supplement will normally result in an increase in milk production, although this is dependent on cow intake. Provision of an energy supplement was shown to reduce urinary and faecal N losses. The effect of timing produced some anomalies, with the 9 h group having lower N losses, but having a reduced level of MS production.

Keywords: supplementation, protein, energy ratio, rumen undegradable protein, rumen degradable protein, timing and synchrony

Many thanks to my supervisors Grant Edwards and Sabrina Greenwood and Dairy Business Centre, for their assistance and support with this project.

A mes beaux parents

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List of Abbreviations

AA	Amino acid
ADF	Acid detergent fibre
BCS	Body condition score
BUN	Blood urea nitrogen
BW	Body weight
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
EAA	Essential amino acid
GE	Gross energy
ME	Metabolisable energy
MicP	Microbial protein
MUN	Milk urea nitrogen
MP	Metabolisable protein
NAN	Non-ammonia nitrogen
NDF	Neutral detergent fibre
NE	Net energy
NH ₃	Ammonia
N ₂ O	Nitrous oxide
NPN	Non-protein nitrogen
OM	Organic matter
PA	Pasture allowance
PDMI	Pasture dry matter intake
PUN	Plasma urea nitrogen
RDP	Rumen degradable protein
RPM	Rising plate meter
SD	Standard deviation
SR	Stocking rate
TMR	Total mixed ration
VFA	Volatile fatty acids

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Chapter 1 Introduction: Objectives, definition of terms and scope of review

The New Zealand dairy herd is undergoing continued genetic improvement, with resultant increases in per cow energy demands and milksolid (MS) production. Due to this, cows in early and mid lactation have a strong homeorhetic drive for high production; at the expense of their body energy reserves (Kuperus, 2003). Although this is true of all dairy cows, higher genetic merit cows will partition a higher proportion of energy available to milk production than lower genetic merit cows. There are several physical constraints to high genetic merit cows satisfying their energy requirements, including: a reduced rumen capacity in early lactation, a limited pasture allowance (PA) and time available for grazing each day and a reduced rate at which ingested material is moved through the rumen (due to the competing processes of passage and digestion) (Kolver, 2003). This results in a reduced ability for the cow to meet her energy demands of lactation. However, meeting these demands may be possible if feeds are fed which are low in neutral detergent fibre (NDF) and high in readily fermentable carbohydrates (RFCHO). Unfortunately, in the NZ pastoral-based system, the feeding of pasture and the grazing conditions imposed by the farmer to maintain high pasture quality through high pasture utilisation come at the expense of achieving this. The purpose of this thesis is to investigate what effect increasing the supply of energy and RUP to the early mid-lactation cow has on MS production and nitrogen (N) partitioning into faeces, milk and urine. Another objective of this thesis is to ascertain what effect partially synchronising the supply of energy and protein availability in the rumen has on MS production and N utilisation.

Higher stocking rates and increased per hectare pasture production requirements are resulting in higher uses of N fertilizer on dairy farms to artificially stimulate increased pasture production. Higher N fertilizer use and higher stocking rates will result in high levels of nitrate discharges onto pasture, which will enter groundwater and eventually contaminate rivers and lakes (NRC, 2007). This is causing an increased need for forages, crops and supplements which lower N losses from the animal or alter N partitioning.

Energy supplementation can be defined as the provision of a source of readily fermentable carbohydrate (RFCHO) with a lower neutral detergent fibre (NDF) concentration than the pasture on offer (NRC, 1985). For grazing dairy cows producing less than 25 kilograms (kg)

of milk per day, the primary nutrient limiting milk production is energy (Kolver, 2003). During lactation, higher input farmers (farmers providing 25% or more of the cows' diet from supplements- Dairy NZ system 5) may provide a supplement that increases the provision of RFCHO to the rumen. Although not strictly considered an energy supplement (owing to its high NDF content), maize silage is widely used in the North Island to provide cows with more energy either during lactation or the dry period. Maize silage is widely used due to its high yield, price competitiveness and flexibility of use in dairy systems throughout lactation. In the South Island energy supplements chosen are predominantly cereal silage and cereal grains. Cereal grains are considered true energy supplements by definition. Cereal grains and cereal silage are cost effective in the South Island (this is not the case in the North Island) and the difficulties and risks associated with growing maize, including lower yields and later frosts, make maize silage an unattractive option in the South Island (Roche and Reid, 2002). Due to the locality of this trial the RFCHO supplement used was the cereal grain wheat. Nutritionally, crushed wheat is high in RFCHO (in the form of starch) and low in crude protein (CP).

After the high energy demands of the early lactation cow have been met the next limiting nutrient for increased milk production is CP (Kolver, 2003). Within the CP fraction of the feed are varying concentrations of rumen degradable protein (RDP), rumen undegradable protein (RUP) and non-protein N (NPN). Rumen degradable protein is broken down by the rumen microbes and incorporated into microbial protein (MicP) (Holmes *et al.*, 2006a). Microbial protein is absorbed from the duodenum after the rumen microbes have completed their lifecycle and travelled to the duodenum (Holmes *et al.*, 2006a). Unlike RDP, RUP is not degraded in the rumen and is instead transported intact to the abomasum, where some of it is degraded to amino acids (AA) that can be absorbed across the duodenal wall (Holmes *et al.*, 2006a). The third fraction of CP is NPN, which consists of free AA, peptides, nitrates, nucleic acids and urea that rumen microbes can utilise for growth (Holmes *et al.*, 2006a). High quality pasture is composed of high concentrations of RDP (>25%) and NPN and low concentrations of RUP. Non-protein N can account for between 20% and 40% of total N in a forage (Waghorn *et al.*, 1996). The RDP and NPN concentrations in pasture are readily degraded (highly soluble) in the rumen and result in pasture N having a high apparent *in vivo* digestibility (approximately 84%) (Kolver and Muller, 1998). However, some researchers believe that an increased provision of RUP will increase protein availability to the tissues (including the mammary glands), leading to increased adipose tissue mobilisation and consequently, increased milk production (Bargo *et al.*, 2002, Jahani-Moghadam, 2009,

Malleson *et al.*, 2008). Others dispute this, claiming that the essential amino acid (EAA) profile of MicP produced from RDP degradation closely matches the EAA profile of milk protein. These researchers believe that providing an RUP source is unnecessary if the RDP requirement of the cow is being met. When feeding a pasture-only diet to cows, the level of RUP in the diet may already be adequate without any supplementation. As pasture is a highly fermentable feed, the rate of passage out of the rumen is high (4%/h to 7%) (Kolver, 2000). This allows a high proportion of protein to escape the rumen undegraded and contributes to the RUP proportion of CP (Kolver, 2000). Therefore, feeding high amounts of good quality pasture may negate any potential benefits achieved from feeding a high-RUP source.

In the literature, high-RUP sources include fish meal, maize gluten meal, expeller soybean meal and blood meal (Bargo *et al.*, 2002). This list excludes canola meal, which was the high-RUP supplement used in the experiment. However, for the purposes of this thesis, a high-RUP source is termed from the NRC (1985) definition, which includes any feed that is fed in such a form as to provide an increase in the flow of AA, unchanged, to the abomasum, yet is made available to the animal in the intestine.

The final objective of this thesis was to ascertain the milk production and N utilisation responses of matching the supply of RFCHO in the rumen more closely with peak rumen ammonia (NH₃) concentrations. In dairy cows, RDP and RFCHO are broken to NH₃ and CHO intermediates in the rumen, respectively (Holmes *et al.*, 2006a). Carbohydrate intermediates are used by the rumen microbes to synthesis VFA (Holmes *et al.*, 2006a). The rumen microbes utilise the NH₃ for growth (Holmes *et al.*, 2006a). However, if CHO substrates are limiting when rumen NH₃ concentrations are high, the excess NH₃ will be absorbed across the rumen wall, where it will be detoxified and excreted via the liver and kidneys, respectively (Holmes *et al.*, 2006a). This is common when feeding pasture as it is rapidly and extensively degraded in the rumen. Gregorini and Soder (2009) performed an *in vitro* rumen simulation study where rumen NH₃ concentrations were reduced by 30% when maize silage was fed 9 h pre-pasture, compared to 1 h before pasture. This marked alteration in rumen efficiency of NH₃ use highlights the shortfalls of providing a diet solely consisting of pasture, with its high concentration of CP. In fact, it is known that pasture-fed cows have an efficiency of N use below 20% (Van Vuuren *et al.*, 1993) and cow faeces contain between two to three times more N than that present in milk (Salter and Slyter, 1974). The cost of detoxifying and excreting this excess N is high (between 0.035 and 0.052 MJME/g N excreted) (NRC, 1989). Kolver and Muller (1998) calculated the cost of excess N removal to be approximately 0.1 kg

MS/d. The environmental effects of excess N provision in the form of CP in dairy cows are also high. The N lost from the cow as urea is converted to NH_3 and N_2O , in the presence of urease (NRC, 2007). Nitrates entering the soil fraction through groundwater contaminate rivers and lakes (NRC, 2007). Nitrogen discharges onto pasture is becoming a pertinent issue for NZ dairy farmers with regional councils in areas in NZ currently putting in place N discharge caps (including the Lake Taupo region).

The objectives of this thesis were to:

1. quantify the MS and N partitioning responses and liveweight (LWT) changes of mid-lactation dairy cows fed a wheat supplement or wheat and canola meal supplement, compared to pasture only
2. quantify the MS and N partitioning responses and LWT changes of mid-lactation dairy cows fed wheat and canola meal either 9 h or 1 h before pasture allocation

The lactation response period that was captured in this thesis was the post-peak lactation period. Full lactation responses were not measured due to time and economic constraints, although there are equations available to extrapolate the likely full lactation responses to the effects of supplementation, based on peak lactation responses.

Chapter 2 Literature Review

2.1 Factors affecting intake in the grazing dairy cow

2.1.1 The New Zealand pastoral system

In New Zealand, dairy farmers receive the lowest farm gate milk prices of anywhere in the developed world (Deane, 1999). As a consequence of this, NZ dairy farmers have developed low-cost farming systems based on grazed pasture, which allows them to compete in world dairy markets. In the 1980's, dairy farmers began to endeavour to make productivity gains through increases in both stocking rate and milk solid production per hectare (Deane, 1999). At this time, most dairy farmers were feeding their cows sole diets of pasture, with less than 20% of farms using supplements (Holmes and Roche, 2007). Over subsequent years farmers realised that additional feed inputs would be required to increase productivity further. This has resulted in a marked increase in supplement use in recent years, with more than 80% of farmers using at least one supplement in 2007 (Deane, 1999; Holmes and Roche, 2007). Other reasons behind increased supplement use include: increased land prices (which have resulted in farmers increasing cow carrying capacity as a means of increasing returns) and an increased proportion of overseas Holstein Freisian (HF) genetics entering the NZ dairy herd (which have a higher homeorhetic milk production drive and as a result of this need to support higher levels of energy intake) (Holmes and Roche, 2007; Kolver, 2003).

2.1.2 Ability and Capacity of the Dairy Cow to Consume Feed

2.1.2.1 *Mechanics of Grazing*

The theoretical DMI of the pastoral fed dairy cow can be calculated according to three considerations. These include bite rate, bite mass and the time spent grazing each day. There is a relatively fixed time cost associated with prehending each bite (Cosgrove and Edwards, 2007). Bite mass is determined by the height and density of the pasture. The time spent grazing per day has a relatively fixed upper limit of 10 to 12 h/d (Holmes *et al.*, 2006a). It is known that total DMI/d will not be sufficient to meet the energy requirements of dairy cows if the sward height drops below 8 to 10 centimetres (cm) (Holmes *et al.*, 2006).

2.1.2.2 *Feed quality*

Feed quality entails the nutritive value (NV) of the feed consumed, the physical characteristics of the feed and its digestibility. The NV of a feed is the concentration of

nutrients contained in the feed and includes both the macronutrient and the micronutrient constituents of the feed. The major macronutrients are structural carbohydrates (NDF), non-structural carbohydrates (RFCCHO), simple carbohydrates (oligosaccharides, disaccharides and monosaccharides), proteins and lipids. Micronutrients include vitamins and minerals. Fibre in well-managed pasture is highly digestible (65% - 75%) (Kolver, 1997) and is degraded quickly (greater than 15%/h) (Burke, 2004).

2.1.3 Pasture Characteristics

New Zealand pastures are commonly comprised of a mixture of perennial ryegrass and white clover. Perennial ryegrass has two stages of growth: vegetative and reproductive. At the vegetative stage the grass species consists of a number of tillers, which are composed of leaf blades and sheaths. At the vegetative stage most of the cell wall components are readily digested. However, as the grass enters a reproductive stage, the proportion of stem material increases and the proportion of leaves and tillers decreases. Leaves have a 70% to 80% digestibility, compared to only 60% to 80% for stem material (Holmes, 1987). Furthermore, from the top tillers of the sward to the stems closest to the ground, the proportion of material that is in the reproductive stage of growth increases (Stockdale, 2001). This effect is due to the upper parts of the pasture being grazed more heavily (Holmes, 1987). Along with these changes there is a concurrent reduction in CP content and an increase in the proportion of NDF. An increase in structural carbohydrates is associated with a fall in digestibility and an increase in the proportion of indigestible residues occupying the rumen (NRC, 2007). These indigestible residues may remain in the rumen until fermentation of the rest of the plant material is complete and will negatively feedback on the hypothalamus, via stretch and tension receptors in the rumen (Waghorn *et al.*, 2007). This effect, combined with the increased amount of time required to chew the fibrous forage, will depress VFI.

2.1.4 Pasture Management

2.1.4.1 Pasture Allowance

Herbage allowance is defined as the amount of herbage above a specified sampling height allocated to livestock (kg DM/ cow/ d). Pasture DMI increases in a convex curvilinear manner from 11.2 kg DM/cow (c)/d up to 18.3 kg DM/c/d when pasture allowance increases from 20 kg DM to 70 kg DM/c/d, with a peak of 55.2 kg DM/c/d (Dalley *et al.*, 1999). Higher pasture allowances enable higher DMI to be maintained but are also associated with reductions in pasture utilisation and quality. Efforts to maintain pasture quality through high pasture

utilisation come at the expense of early and mid lactation cows meeting their energy demands, without supplementation this may result in either reductions in animal output or excessive losses of body condition, through LWT mobilisation.

2.1.4.2 Pasture Utilisation

A low utilisation of pasture (often associated with higher pasture allowances) will result in a deterioration of pasture quality as the season progresses due to an increase in the residual pasture height (Butler *et al.*, 1988). Pasture height is a major factor determining pasture utilisation in dairy cows as cows are known to consistently remove approximately one third of the pasture height on offer, regardless of pasture height (Gibb *et al.*, 1997). Gibb *et al.* (1997) observed that when dairy cows were continuously grazing perennial ryegrass pasture, bite mass decreased from 0.31 g organic matter (OM)/bite at a height of 9 cm to only 0.23 g OM/bite at a height of 5 cm. This effect was seen to occur without changes in either biting rate (76 bites/minute (m) or grazing time (604 m/d). These findings disagree with those found by Hodgson and Brookes (1999), who found grazing time and bite rate to act as partial compensatory mechanisms that increased when bite mass decreased.

The net growth of the grass sward occurs between the heights of 3 and 6 cm (Hodgson and Jamieson, 1981). However, 3 to 6 cm is considered too low for cows to meet their ME requirements and a more practical sward height for grazing cows is thought to be between 10 and 12 cm. This means a compromise must be reached between the productivity of the sward and the maintenance of high herbage intakes.

2.2 Energy Supplementation

2.2.1 Justification for supplementation

There are inherent stresses involved with high genetic merit cows achieving high MS yields when consuming pasture in large herds. There are also periods throughout the year when pasture growth is inadequate to meet animal demands for nutrients. The composition of pasture, with its relative excess of N to carbon precursors can lead to reduced microbial protein (MicP) synthesis which can lead to reduced milk production responses being seen in grazing cows (Schroeder *et al.*, 2004). Furthermore, the NZ dairy herd is currently undergoing marked improvements in genetic capabilities for increased milk production. In the last 31 years the genetic potential for milk production of the average NZ dairy cow has increased by 20% (Macdonald *et al.*, 2008b). The average breed of the NZ dairy herd was predominantly Jersey in 1980 and is now Jersey x Friesian, with a resultant increase in LWT from 390 kg to

460 kg (Macdonald *et al.*, 2008b).

Higher land and operating costs on farms in recent years (Kolver, 2003) have resulted in higher stocking rates (SR). Between 1980 and 2010, SR have increased on NZ farms from 2.1 cows/ ha in 1980 to 2.8 cows/ ha in 2010 (LIC, 2010). This is due to higher SR allowing higher per hectare milk production (Macdonald *et al.*, 2008a). A farm with 390 kg cows and a 2.1 cow/ha SR would require 10.6 T DM/ ha grown/ year (Macdonald *et al.*, 2008a). However, today's average cow LWT and SR require a yearly pasture growth of 16.7 T DM/ ha (Macdonald *et al.*, 2008a). Historically, this pasture harvest was achieved in only 5.3% of dairying regions of NZ in 2010 (DairyNZ, 2010). This therefore necessitates either reducing SR or including external feed sources into the farming system. NZ farmers are choosing to add supplementary feeds into their system to manage this deficit (Kolver, 2003). The choice of supplement depends on the purchase price, labour, machinery and equipment requirements. In the South Island of New Zealand, a number of farms are installing in-dairy feeding facilities to provide cereal supplements during lactation (de Ruiter *et al.*, 2007).

2.2.2 Effects of energy supplementation

2.2.2.1 Milk production

Milk production is known to increase as the level of concentrate feeding increases (Delaby *et al.*, 2001). The response in milk production to energy supplementation is partially due to the increase in total DMI, as there is known to be a positive relationship between milk production increase, concentrate DMI and total DMI consumed (Bargo *et al.*, 2003).

2.2.2.2 Milk composition

Many studies have shown that an increased level of concentrate in the diet will reduce milk fat concentration (Peyraud and Delaby, 2001). Concentrate supplementation has also increased the protein content of milk in some studies (Bargo *et al.*, 2002, Delaby *et al.*, 2001), while not in others (Carruthers *et al.*, 1997). An increased level of milk production through concentrate supplementation may accompany an increased protein content in the milk, due to an improved metabolic status of the cow (Peyraud and Delaby, 2001).

2.2.2.3 Nitrogen utilisation

When pasture is consumed, RDP and RFCHO are broken down to ammonia (NH₃) and carbohydrate intermediates, respectively (Holmes *et al.*, 2006a). Carbohydrate intermediates are used by the rumen microbes to produce volatile fatty acids (VFA) (Holmes *et al.*, 2006a).

The rumen microbes utilise the NH_3 for protein synthesis (Holmes *et al.*, 2006a). However, if energy substrates are limiting when rumen NH_3 concentrations are high, the excess NH_3 will be absorbed across the rumen wall, where it will be detoxified and excreted via the liver and kidneys, respectively (Holmes *et al.*, 2006a). Some of this NH_3 will be released from the animal and will volatilise into N_2O , which is a greenhouse gas. This is common when feeding pasture as it is rapidly and extensively degraded in the rumen. Boudon and Peyraud (2001) found the N: RFCHO ratio of grazing dairy cows to be between 40 and 129 g N/RFCHO kg DM. Lincoln University dairy farm in 2010/2011 had an average N: RFCHO ratio of 148 g N/kg RFCHO (SIDDC, 2010). Therefore, rumen fermentation of N is less than optimum and the efficiency of N use is low in pastoral-fed cows (<20%) (Van Vuuren *et al.*, 1993). Cow faeces usually contain between two to three times more N than that present in milk (Salter and Slyter, 1974). The cost of detoxifying and excreting the excess N in the urine is high (between 0.035 and 0.052 MJME/g N excreted) (NRC, 1989). When a source of RFCHO enters the rumen when the NH_3 concentration is high, the utilisation of the NH_3 present will be increased and less NH_3 will be absorbed across the rumen wall.

2.2.2.4 Substitution and body condition score

Substitution refers specifically to the reduction in pasture intake (kg DM/c/d) that occurs for each kg DM supplement consumed (Stockdale, 2001). When a feed is supplemented that has a lower NDF content than the forage on offer, the cow will reduce her intake of the forage. Substitution rates vary between 0.2 kg DM pasture substituted per kg DM concentrate at very low levels of supplementary feeding to 0.8 kg DM pasture substituted per kg DM concentrate at very high levels of supplementary feeding (Holmes, 1999).

A dairy cow is capable of producing between 130 and 140 g MS from the amount of energy contained in one kg DM of wheat. However, short-term responses to supplementation are usually only 27% of the theoretical response expected (Penno, 2002). Long-term responses seen in the literature are higher than this and range between 60 and 100 g MS/kg DM (Holmes, 1999). The reduced responses seen, compared to the theoretical responses predicted, are partially due to the substitution effect that supplements have on pasture intake and also due to the diversion of some of this extra energy to other bodily processes (Meijs and Hoekstra, 2006).

Lax grazing of the sward due to substitution will suppress white clover growth and increase the proportion of grass stem and dead material in the forage (Butler *et al.*, 1988). This

facilitates fungal incursion, decreases the digestibility of the white clover and increases the amount of mechanical chewing required to digest the forage (Butler *et al.*, 1988).

2.2.2.5 Diet digestibility

A further consideration when supplementing cows with energy concentrates is the reduction in fibre digestion that may occur. Cellulolytic bacteria preferentially digest starch and will reduce the functioning of other fibre digesters in a high-RFCHO diet, through a reduction in pH (below the critical lower limit of 6.2) (Stewart and Bryant, 1988). The population of amylolytic bacteria (starch digesters) will increase if a high starch diet is introduced. Amylolytic bacteria rapidly degrade starch and cause rapid reductions in rumen pH. Amylolytic bacteria have a faster growth rate than cellulolytic bacteria and therefore proliferate faster, perpetuating the negative effect that the reduced pH can have on cellulolytic microbes (Van Soest *et al.*, 1988). The reduction in fibre digestion when a high starch diet is fed can be marked. Opatpatanik *et al.* (1995) found that wheat supplementation inhibited NDF digestion by 15% when fed with perennial ryegrass at a ratio of 75% forage: 25% concentrate. The amount of time that pH is suboptimal is more detrimental to fibre digestion than mean rumen pH (Opatpatanakit *et al.*, 1995). Increasing the period of exposure to a suboptimal rumen pH will reduce the concentration of acetate and total volatile fatty acids (VFA) formed in the rumen (Russell, 1998).

2.3 Rumen Undegradable Protein Supplementation

2.3.1 Justification for supplementation

The primary nutrient limiting milk production when the level of production is less than 30 L/d is energy availability (Kolver and Muller, 1998). When energy requirements have been met the next limiting nutrient in high producing dairy cows is CP (Kolver, 2003). The protein available to the animal is a combination of MicP, endogenous protein and undigested feed protein which has escaped rumen microbial digestion. Although pasture provides sufficient RDP, it is postulated by some to be deficient in RUP (Malleson *et al.*, 2008). A high yielding dairy cow in early lactation requires between 16% to 17% CP on a DM basis, with approximately 37% to 38% of this CP being in the form of RUP (6.5% - 7.2% on DM basis) to optimise milk production (Hongerholt and Muller, 1998). As pasture provides less RUP than this recommendation (4.9% DM) it would be thought that RUP supplementation would increase animal performance. To date however, the effect of RUP supplementation on milk production under grazing conditions has only been investigated in a few studies with high producing cows (Schor and Gagliostro, 2001).

One high-RUP source is canola meal. Canola meal is a by-product from the mechanical processing of the canola seed for oil production. The heat production that occurs when canola undergoes the extraction process can reduce the degradability of the CP in the rumen (Orskov, 1992). In doing so, heat processing increases both the RUP and indigestible protein fractions of canola (Orskov, 1992).

2.3.2 Amino acid use

Metabolisable protein (MP) in the form of AA that are absorbed through the ileal wall, constitutes all of the protein available to the animal (Nicol and Brookes, 2007). During passage across the intestinal wall, some AA are incorporated into intestinal proteins (constitutive or secretions) or catabolised by intestinal tissues (limited to non-essential AA) (Atasoglu and Wallace, 2003). Despite only comprising between 4 and 8 percent of whole body protein mass, intestinal tissues account for between 20% and 35% of whole body protein synthesis in ruminants (Lapierre *et al.*, 1999). Following intestinal use, the remaining AA will be transported to the hepatic tissue where they have one of three fates. These include: passing directly through and being made available to peripheral tissues; being extracted for synthesis of proteins, constitutive of liver matrix or exported as plasma proteins; and being oxidised within the liver with the N being potentially lost as urea. These three metabolic fates must be coordinated to ensure the supply of AA to peripheral tissues is adequate, whilst not reaching toxic levels. This usually comes about through obligatory losses of AA through hepatic oxidation (Bequette *et al.*, 2003). The post-liver supply of the essential AA: His, Met and Phe are nearly equal to their net mammary uptake and secretion into milk protein (Lobley and Lapierre, 2001). Furthermore, if the post-hepatic supply of AA is increased, there will be increased uptake by the mammary gland (Bequette *et al.*, 2003). The AA transporters in the mammary gland express both saturation kinetics and a non-saturable diffusion component (Bequette *et al.*, 2003). The transporters on the cell membrane are also known to be bi-directional (Baumrucker, 1985). If the supply of non-essential AA to the mammary gland is in excess, these can be catabolised and converted to other limiting AA. However, if the supply of essential AA is limiting, milk production efficiency will be impaired.

Therefore, not only is the total amount of RUP in the diet important for determining milk production response, but the specific EAA profile of the RUP supplement may also be important. Lysine (Lys) and Methionine (Met) are the first and second limiting AA for milk production, respectively (Kolver, 2003, Richardson and Hatfield, 1978). Santos *et al.*, (1998)

reviewed 88 studies where either fish meal or soybean meal was supplemented and found fish meal to increase milk production more significantly than soybean meal. This effect was seen to occur despite the fact that soybean meal has a higher RUP concentration than fish meal. Fish meal has a higher EAA index than soybean meal and this indicates that the EAA profile of the RUP source is more important than the total amount of RUP. The amount of Lys and Met required as a percentage of total EAA in the duodenal digesta for milk production is 16% and 5%, respectively (Schwab, 1994). This has important implications for potential supplementary RUP sources, as it can be seen from Table 1 that most RUP sources available in NZ (excluding fish meal) have Lys and Met profiles that are inaccurately matched to milk protein.

Table 1 Lysine and methionine percentage of total EAA in milk protein, rumen bacteria and some common high-RUP supplementary feeds, adapted from Schwab (1994)

	Lys % EAA	Met % EAA	EAA % CP
Milk protein	16.4	5.1	38.4
Rumen bacteria	15.9	5.2	33.1
Fish meal	16.9	6.5	44.8
Blood meal	17.5	2.5	49.4
Soya bean meal	13.8	3.1	47.6
Canola meal	17.0	3.1	38.0

Lys- Lysine; EAA- essential amino acid; Met- Methionine; CP- crude protein

2.3.3 Effects of rumen undegradable protein supplementation

2.3.3.1 Milk production

A review by Bargo *et al.* (2003) reported a mean increase of 0.8 kg MS/d for each 100 g/d RUP in an iso-energetic diet. Malleson *et al.* (2008) performed a study with 45 multi-parous Jersey cows where a high-RUP source was fed at 4% of DMI and found milk production to significantly increase. Jahani-Moghadam (2009) performed a trial in early lactation dairy cows where soybean meal (low-RUP) or xylose-treated soybean meal (1.57 kg/d RUP) was fed and found the xylose-treated soybean meal to increase milk production by 3.2 kg/d, from 29.7 kg/d to 32.9 kg/d. Santos *et al.*, (1998) conducted a trial where treated canola meal with a RUP percentage of 43% was fed to early lactation cows and found milk production to increase significantly by 3.8 kg/d. Whitelaw (1987) also investigated the theory that increased RUP increases milk production. Whitelaw (1987) noted that when casein was infused into the abomasum of cows, adipose tissue mobilisation increased significantly. It was proposed that when early lactation cows are provided with a high-RUP source, BW mobilisation is stimulated and this provides the glucose precursors needed for increased milk production.

Cows with more adipose tissue at calving may also have a higher requirement for RUP, due to their reduced feed intakes governed by appetite (Stockdale, 2001).

In a review of 88 lactation trials performed over 12 years (using predominantly soy bean meal) Santos *et al.* (1998) observed that only 17% of the trials found significantly higher milk production when a high-RUP source was fed. Stockdale (2008) found no significant difference in milk yield between cows fed treatment diets of untreated canola meal and heat-treated canola meal. This lack of response may be due to decreased microbial rumen synthesis (Schwab, 1994), low small intestine digestibility of RUP (Schwab, 1994), or feeding a control diet that was already sufficient in RUP (NRC, 1985).

2.3.3.2 Nitrogen utilisation

Canola meal is high in RUP (35.7% of CP) compared to pasture (12.3% of CP). Canola is also high in RFCHO (31.1% DM) compared to pasture (12.3% DM). The relatively higher concentration of RFCHO in canola meal, compared to pasture, could improve N utilisation when canola meal is fed. This effect would be due to the additional RFCHO in the canola meal utilising a higher proportion of the NH_3 in the rumen.

2.3.3.3 Body condition score

Schei *et al.* (2005) found that increasing the amount of RUP increased adipose tissue mobilisation, pushing cows into a greater negative energy balance. From this it can be extrapolated that feeding a high-RUP source to a cow with a higher body condition score (BCS) cow at calving will result in a greater milk production response than if the RUP source was fed to a cow with a lower BCS (Garnsworthy, 1989). Other researchers have failed to find significant liveweight (LWT) differences between cows fed low-RDP versus high-RUP diets, including Orskov *et al.* (1981).

2.4 Timing of Supplementation

2.4.1 Justification

It has been postulated that timing the release of RFCHO in the rumen to match peak rumen NH_3 concentrations will increase N utilisation. This has been predicted to allow more efficient microbial capture of N (MicP production) and increase the efficiency of use of ATP for VFA production. Without rumen synchrony in high RFCHO diets, it is thought that N supply to the rumen microbes will be deficient and fermentation will occur largely without microbial growth. However, some researchers have found that synchronising energy and protein

availability in the rumen does not affect feed utilisation efficiency (Trevaskis *et al.*, 2004).

Dairy cows on 24 h pasture breaks consume the bulk of their pasture (>70%) in the first 3 to 4 h after allocation (Trevaskis *et al.*, 2004). Also, grazing cows are known to consume more DM when offered their new pasture allocation in the afternoon (Rook *et al.*, 2004). This is because the concentration of water soluble carbohydrates (WSC) increases in the sward throughout the day and reaches a peak in late afternoon (Hoover and Stokes, 1991). Hoover and Stokes (1991) observed the WSC content of pasture to be 52 g/kg DM higher in the afternoon, than in the morning. Miller *et al.* (2001) found PM perennial ryegrass-based pasture (165 g/kg DM WSC) to increase milk yield from 12.6 to 15.3 kg/c/d, compared to a.m. perennial ryegrass-based pasture (126 g/kg DM WSC). Authors in support of this theory of matching rumen NH_3 and energy availability have put forward the idea that the most efficient use of dietary N and RFCHO will take place when the high-RFCHO source enters the rumen 7 to 9 h after a grazing bout, when peak rumen NH_3 concentrations occur (Soriano *et al.*, 2000). Therefore, allocating cows their fresh pasture break following the afternoon milking will theoretically maximise DMI and diet digestibility and could carryover to increased MS production, compared to other pasture feeding strategies.

2.4.2 Effects of timing the release of energy and protein in the rumen

In a study performed by Trevaskis *et al.* (2004) using grazing dairy cows allocated a high-RUP supplement, no milk production benefit was observed when supplementation occurred 12 h before pasture allocation. The cows that were given their supplement 1 h before pasture allocation produced significantly more milk than cows fed their supplement 12 h before pasture allocation (25.1 versus 24.3 kg/d, respectively). It was interesting to note that the third treatment group, who were given their supplement 10 h before their evening pasture allocation, had a significantly higher mean milk yield than either of the two other treatment groups (26.8 kg/d). This effect was thought to be due to higher DM digestibility (+4%) in the evening pasture, owing to its higher concentration of WSC.

There has only been a limited amount of research to date looking at the effects on N utilisation of the timing of supplementary feeding, this includes a study by Mitani *et al.* (2005) and a study by Gregorini and Soder (2009). Mitani *et al.* (2005) observed greater milk N output in cows fed a maize silage supplement pre-grazing, compared to those fed the same supplement post-grazing. Gregorini and Soder (2009) used a dual-flow continuous culture fermentor to test the effects *in vitro* of providing maize silage either 1 h or 9 h before pasture.

The researchers found rumen NH_3 concentrations to be reduced from 11.4 mg to 8.8 mg NH_3 /100 mL rumen fluid, when the high-RFCHO source was given 9 h before pasture intake. This equated to a 30% reduction in rumen NH_3 concentrations, compared to when the high-RFCHO was fed 1 h before pasture intake. It was also found that the glucogenic precursor: propionate, increased in concentration by 13% in the 9 h treatment.

2.5 Conclusions and Objectives

2.5.1 Conclusions

The NZ dairy herd is currently undergoing a marked shift in genetics, with an increase in the proportion of NA HF genetics. These NA HF cows require an increased supply of energy to meet their increased energy demands in lactation. Lactating cows have many constraints to satisfying their daily energy requirements, including: a limited time available for grazing each day and a reduced rate at which ingested material is moved through the rumen (due to the competing processes of passage and digestion and a reduced rumen capacity). One possibility open to farmers for meeting the energy requirements of lactating cows of high genetic merit is to offer feeds which are low in NDF and high in RFCHO. Unfortunately, in the NZ pastoral-based system, the feeding of pasture and the grazing conditions imposed by the farmer to maintain high pasture utilisation and quality do not allow this to occur. This has paved the way for increased supplement use on farms, with more than 80% of farmers using some form of supplement in 2007 (Holmes and Roche, 2007).

Improved milk production worth (PW) genetics of the NZ dairy herd is resulting in increased feed requirements per cow and higher milk production yields. Meeting these high energy demands with pasture is difficult in early and mid lactation due to a necessity to maintain high pasture quality and utilisation. This has led to some farmers using high-energy supplements to meet the high energy demands of their cows. However, much debate has occurred in NZ in the past regarding the place of supplements in NZ's traditional low-cost/ low-input system (Roche and Reid, 2002).

If energy supplements are used, the next limiting nutrient to increased milk production is protein supply (Kolver, 2003). However, research that has been carried out to date regarding high-RUP supplementation in a pastoral-based setting has produced conflicting results (Bargo *et al.*, 2002). This is also true of research relating to the timing of supplementation relative to pasture allocation (a concept commonly referred to as synchrony). Therefore, there is strong

justification for conducting an experiment where the effects of providing a high-RUP source and altering the timing of concentrate: herbage allocation is measured.

2.5.1.1 Energy supplementation

Improved milk production worth (PW) genetics of the NZ dairy herd is resulting in increased feed requirements per cow and higher MS yields. Meeting these high energy demands with pasture is difficult due to a necessity to maintain high pasture quality and utilisation. This has led to some farmers using high energy supplements to meet the high energy demands of their early lactation cows. However, much debate has occurred in NZ in the past regarding the place of supplements in NZ's low-input system (Roche and Reid, 2002).

2.5.1.2 Rumen undegradable protein supplementation

If energy supplements are implemented into a feeding strategy, the next limiting nutrient for increased MS production will be CP availability (Kolver, 2003). A form of CP that is directly available to the animal without breakdown in the rumen is RUP. However, research that has been carried out to date regarding RUP supplementation in a pastoral-based setting has produced conflicting results (Bargo *et al.*, 2002).

2.5.1.3 Timing of supplementation

There may be MS and N utilisation benefits achievable from providing a source of RFCHO to the rumen at the time of peak rumen NH_3 concentrations. However, when using 24 h breaks in the p.m. (to gain maximum WSC); supplement allocation would have to take place between 0000 h and 0200 h. This is not feasible for most farmers, alternative options include: foregoing the benefit of the additional WSC by feeding pasture in the a.m. and supplementing in the p.m. (supplementation occurring 10 h post grazing); or feeding the supplement around the time of the a.m. milking (supplementation occurring 14 h post pasture allocation). For this experiment it was chosen to supplement 14 h after pasture intake (9 h pre-grazing), due to the increased WSC concentration.

2.5.2 Hypotheses

1. Supplementing grazing dairy cows with a high-RFCHO source is predicted to simultaneously increase milk production and improve N utilisation, above pasture-fed cows.
2. Providing the cows with a supplement high in RUP is not predicted to result in any further increases in either milk production or N utilisation, above either pasture-fed or wheat-supplemented cows.
3. Timing the supplementation to coincide more closely with peak rumen ammonia concentrations (9 h treatments) is expected to increase milk production and N utilisation, above that in the 1 h treatment groups.

In summary, providing a concentrated energy supplement to the cows, regardless of type, is expected to increase milk production and N utilisation. However, if the amount of energy in the diets of the high-RUP treatments is kept constant, it is predicted that no further increases in either milk production or N utilisation will be seen through providing a higher fraction of RUP. In addition, matching up RFCHO supply more closely with peak rumen ammonia concentrations (9 h treatments) is expected to have beneficial effects on both MS production and N utilisation, over the 1 h treatments.

2.5.3 Specific Objectives

1. To determine the differential effect on MS yield of feeding wheat and canola meal at 9 h and 1 h intervals before pasture allocation; compared to feeding pasture
2. To determine the differential effect on urinary and faecal N losses of feeding wheat and canola meal at 9 h and 1 h intervals before pasture allocation; compared to feeding pasture

Chapter 3 Materials and Methods

This experiment was carried out under the authority of Lincoln University Animal Ethics.

3.1 Location and Duration

The experiment was conducted at the Lincoln University Research Farm (LURF), Lincoln, New Zealand between 25 October and 26 November 2010. The total rainfall in Lincoln in November 2010 was 50.6 millimetres (mm). The mean daily temperature during the experiment was 19.6° C (1 November – 25 November, 2010) (Lincoln Meteorological Station, Lincoln).

The cows were grazed on 6.0 ha of land on a Templeton silt loam/ sandy loam soil. The pasture used for the study was a mixture of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens*), established 18 months before the experiment began. The trial area was fertilised with 50 kg N/ ha four weeks preceding the experiment and was not fertilised during the experimental period.

The selection of cows used in the experiment was performed on the 20 September, 2010. Measurements were taken from the 18 October 2010 to 26 November 2010.

3.2 Cows and Experimental Design

A total of 30 spring calving, Jersey x Friesian, multi-parous cows from the LURDF herd were blocked according to: BW (457 ± 44); milk yield (23.0 ± 4.6); parity (4 ± 2); days in milk (52 ± 11). These cows were then allocated at random to five treatment groups of six cows per treatment, with the experimental units being the cows. The cows underwent a 7 d adjustment period before undergoing a 24 d treatment period. One cow in the E1 treatment group had to be excluded from the experiment during the transition period and was not replaced, due to a total refusal to eat any supplement offered.

3.3 Treatments

The five treatment groups tested represent a control group (pasture only) and factorial combinations of an energy supplement offered 1 or 9 hours before pasture, either offered alone or with RUP supplementation. Specific details of the treatments are given below:

C: 17.0 kg DM pasture/c/d (above a post-grazing residual of 1480 kg DM/ ha) allocated after the evening milking.

E9 (energy concentrate allocated 9 h before pasture): 11.5 kg DM pasture (above a post-grazing residual of 1480 kg DM/ ha) and 5.1 kg DM wheat allocated at 1700 h and 0600 h, respectively.

E1 (energy concentrate allocated 1 h before pasture): 11.5 kg DM pasture (above a post-grazing residual of 1480 kg DM/ ha) and 5.1 kg DM wheat allocated at 1600 h.

P9 (RUP concentrate allocated 9 h before pasture): 11.3 kg DM pasture (above a post-grazing residual of 1480 kg DM/ ha) allocated at 1700 h and 1.8 kg DM wheat and 3.0 kg DM canola meal allocated at 0600 h.

P1 (RUP concentrate allocated 1 h before pasture): 11.3 kg DM pasture (above a post-grazing residual of 1480 kg DM/ ha), 1.8 kg DM wheat and 3.0 kg DM canola meal allocated at 1600 h.

The C and E diets were designed to be iso-energetic (212.5 MJME) and differed in the concentration of CP and the fractions of RDP and RUP. The P diets were iso-nitrogenous with the C diet (26.5% CP) and differed only in the proportion of RDP and RUP. The cows were adjusted to their treatment diets from their baseline diets over the first 7 days, with a 15% change in diet occurring per day.

Canola meal was the high-RUP source used in this experiment based on its availability and current use in NZ dairying, as opposed to the other aforementioned high-RUP feeds, which were either not in use in NZ at the time of selection (fish meal, maize gluten meal and blood meal) or were more expensive than canola meal (soybean meal).

3.4 Management

The cows strip-grazed the pasture and were moved to a new grazing break at approximately 1700 h, once a day, after the afternoon milking. The cows were allocated their new break in the afternoon to coincide with the peak in WSC that was known to occur in late afternoon. Portable electric fences were used for back-fencing each group in the area allocated for that 24 h. The cows grazed all hours (except for the milking and supplementation times) and clean

drinking water was available from portable troughs *ad libitum*. The cows had a 15 d rotation during the trial and re-entered the same paddock once during the experimental period. To replicate the management practices that would occur in a real farming situation where supplementation is used, the cows were expected to re-enter the paddock that they grazed last rotation without any measures taken in the interim to ensure post-grazing pasture mass was maintained close to 1480 kg DM/ha. This is because substitution of pasture when supplementing is a genuine management issue on farm and it was intended to attempt to look at the effects of supplementation on a whole-farm level.

The cows were milked twice daily at 0700 h and 1500 h. The mean walking distance to the milking parlour was 0.38 km (range 0.21 to 0.55 km). The concentrates were offered individually to cows in feed bins located adjacent to the milking parlour and any uneaten feed after 1 h was collected and weighed.

Figure 1 Method by which the control treatment group were drenched daily



A plastic 300 ml bottle containing 77 g of NaHCO_3 diluted in 250 ml water was forced part way down the throat of each cow until all of the liquid had been swallowed. This vet race was also used for animal sampling.

The cows were adapted to the new diets for the first 7 days (increase of 15% of DM target per day). This slow transition to the experimental diets was considered essential to modify the rumen microbial population before the intensive experimentation period began. During the adjustment period, the cows were trained to eat the concentrates under two different time regimes. On d 0 the cows were allocated 100% of their diet as pasture. On d 1 the treatment cows were allocated diets consisting of 15% target maximum supplement diet (either wheat or wheat plus canola meal): 85% non-treatment diet. During the next six days the composition of the diet was altered by an additional 15% of the target diet per day. By d 7 the treatment cows were receiving their full treatment diet.

Figure 2 P9 group eating their supplement from the lockable bales and bins.



Figure 3 View of P9 cow eating out of bottom of bin.



3.5 Measurements

3.5.1 Pasture

3.5.1.1 *Calibration of the rising plate meter*

Calibration of the rising plate meter (RPM) (Jenquip F150 Electronic Pasture Meter) was necessary as the weight per unit area under the plate would have affected the quantifiable relationship between the height and yield of the pasture (Earle and McGowan, 1979). Four low, low-medium, medium, medium-high and high pasture heights were selected to calibrate the RPM. The pasture height was measured with the RPM at these sites (0.2 m^2) and the grass under the plate was cut to ground level. Each sample was then weighed and dried at 60° C for 72 h to determine the pasture yield (kg DM/ ha). This was done every week and all of the data was computed, with a total of 86 calibration cuts taken during the experimental period.

The LINEST function in Microsoft Excel was used to fit the data to a linear regression equation using the pasture masses and RPM readings. The regression equation used was $Y = aX + b$, where: Y = pasture mass (kg DM/ha) and X = RPM reading (Hodgson and Brookes, 1999). This regression equation could then be used to estimate DM yield at a given RPM reading.

The calibration cuts were completed prior to the experimental period and the regression equation used was yield/ha (kg DM/ha) = RPM height * 105 + 63 ($r^2=0.68$). Once the yield/ha had been estimated, the area needed for a pasture intake of approximately either: 17, 11.5 or 11.3 kg DM/c/d was calculated, based on a desired post-grazing residual of 1480 kg DM/ ha.

3.5.1.2 Estimating pasture allowance and intake using the rising plate meter

Pasture dry matter intake (PDMI) was estimated as a group, as estimating individual cow intake has inherent difficulties (Stewart *et al.*, 1995). Pasture dry matter intake was estimated from the difference method using pre- and post- grazing measurements from the rising plate meter (Kellaway *et al.*, 1993). Stockdale and King (1983) compared animal-based techniques to the difference method for predicting PDMI and found the difference method to be more accurate as animal production based on DMI was closer to the predicted values. The pasture height was estimated by taking 20 RPM reading on each grazing pre- and post- grazing strip to determine mean pasture height.

3.5.1.3 Chemical composition

Every Monday and Friday, two pre-grazing pasture samples were taken for each treatment group. This took place at 0900 h and 1500 h (25 October to 26 November 2010). The herbage was plucked with shears from the grazing horizon (3 cm above ground level or higher) along a zigzag transect through the paddock. Three plucks were obtained from six sites at each paddock to estimate the NV of the grazed portion of the canopy. The pluck samples from each treatment paddock were then thoroughly mixed. The samples were freeze-dried at 60° C for 72 h and then ground through a 1 mm screen to determine DM, CP, WSC, NDF, ADF and DOMD concentration using near infrared spectroscopy (NIRS) (NIRS Systems, 2010).

3.5.1.3.1 Rumen undegradable protein content of pasture

The RUP proportion of the pasture was estimated by performing a neutral detergent digestion on the ground forage samples and analysing the residual CP remaining by NIRS (NIRS Systems, 2010).

3.5.1.4 Botanical composition

The botanical composition of the pre- and post- grazing area in each treatment paddock was taken twice weekly on Monday and Friday (25 October 2010 to 26 November 2010). A bulk sample of 18 plucks along a zigzag transect through the paddock were taken and these were mixed, quartered, dissected into components, dried at 65 °C for 72 h and then weighed to give

botanical composition on a dry weight basis. Dissected components included: grass leaf, grass stem, grass reproductive tillers, legumes, weeds and dead material.

3.5.2 Concentrate samples

Bulk concentrate samples were collected before the start of the experiment (20 September 2010) by taking four sub-samples from within the concentrate and mixing these to obtain one concentrate sample per supplement. The samples were milled to 1 mm and then measured for DM, CP, crude fat, NDF, ADF, MJME/ kg DM and DOMD contents using the following methods: DM was determined gravimetrically (fan-forced oven, 100°C, 48 h, constant weight); CP was measured by combustion of the samples under oxygen supply and high temperatures using a Variomax CN analyser, Elementar; crude fat was measured by soxhlet extraction in hexane using a Tecator Soxtec 1043 (FOSS, 2010); NDF and ADF were measured by the method of Van Soest *et al.* (1991); MJME was measured with the Foss Feed and Forage Analyser, 5000 (FOSS, 2010); and DOMD was assayed by an *in vitro* pepsin cellulose method, corrected by use of *in vivo* standards.

Table 2 Chemical composition of the supplements used

Chemical component	Concentrate	
	Wheat	Canola meal
DM %	88.0	93.5
NDF, % DM	10.3	20.8
ADF, % DM	3.2	17.4
RFCHO, % DM	73.7	31.1
Fat, % DM	1.5	9.7
CP, % DM	12.5	35.7
RDP, % CP	57.0	64.3
RUP, % CP	43.0	35.7
Lys, % EAA ¹	6.6	13.2
Met, % EAA ¹	5.2	4.4
DOMD	100.0	83.7
MJME	13.7	14.4

¹ Estimated from NRC (2001); WSC- water soluble carbohydrate; DOMD- degradable organic matter digestibility

The NDF % and DOMD of the canola meal were higher and lower, respectively, than wheat. A higher NDF % and lower DOMD would normally represent a feed of lower NV. However, owing to the higher proportion of CP and fat in canola meal, the MJME/ kg DM value was higher and the canola meal was therefore considered a higher quality feed than the wheat. The RUP % of CP in the canola was only marginally higher than that of wheat. However, because the CP % of DM was much higher in the canola, the amount of RUP provided per kg DM of supplement was higher in the canola.

3.5.3 Milk production and composition

The cows were milked in a 12-aside herringbone shed with electronic milk meters (DeLaval Alpro Herd Management System) twice daily at 0700 h and 1500 h. The daily milk yield (l/cow) of the cows was measured in the milking parlour and automatically recorded. Daily milk yields were obtained by summing the p.m. and following a.m. yields. The mean milk production for the experimental period for each cow (25 October – 26 November 2010; after the continued feeding period) was calculated.

Two milk samples were taken on every sampling day (18 October, 1 November, 8 November, 15 November, 22 November and 25 November 2010), with one sample being transported to LIC (Livestock Improvement Company, 2010) and the other sample refrigerated for approximately 1 h at 8° C. The refrigerated sample was sub-sampled into a centrifuge tube and centrifuged at 3500 x g for 10 min at room temperature (22° C), before being refrigerated for a further 10 min to solidify the fat layer. After 10 min, the fat layer was removed and a subsample of the skim milk was pipetted into a clean microcentrifuge tube and this skim milk sample was frozen at -20° C. The sample was later measured for MUN content with the Enzymatic Kinetic UV assay using the Randox Kinetic Kit (Randox Rx Daytona, 2010). The MUN was calculated as the molar concentration of milk urea multiplied by two. The second sub-sample sample was analysed for fat, lactose and protein percentages at LIC (Livestock Improvement Company, 2010) using the Milk-o-scan infrared analyser (Foss Electric Ltd.).

3.5.4 Body weight and body condition score

The cows were weighed as they left the milking parlour after each milking during the experimental period. On measurement days (18 October, 1 November, 8 November, 15 November, 22 November and 25 November 2010) a second set of scales (Tru-Test) was used to weigh the cows.

The BCS recorded prior to the start of the experiment of the cows was used to block the cows into treatment groups and was determined by palpitation of the back and hind-quarter areas, with a score of 1 to 6 was given (where 1 was thin and 6 was obese using the scale outlined by Roche *et al.* 2009). The condition scoring was performed on the 28 September and the 22 November 2010 by Laura Rossi, Dairy NZ.

3.5.5 Faecal samples

Faecal samples were taken twice daily (0800 h and 1600 h) from each cow on the 18 October, 1 November, 8 November, 15 November, 22 November and 25 November 2010. Samples were gathered in 250 ml plastic containers after voluntary defecation or after stimulation of defecation by rubbing the rectal wall, and faecal samples were then brought back to the lab and frozen at -20° C. The samples were later defrosted and mixed, before taking two sub-samples. One sub-sample was weighed in a tin petri dish and dried at 80° C for 72 h and then reweighed to calculate faecal DM. The second sub-sample was deposited into a 70 ml plastic container and dried in a forced-air drying oven for 48 h, before being ground through a 1 mm screen to reduce particle size and ensure uniformity of particle dimension. The dried sample was then analysed for N content in the LU lab by combustion under oxygen supply and high temperatures using the Variomax CN Analyser; Elementar.

Estimations of faecal DM and total DM intake allowed apparent *in vitro* organic matter digestibility (IVOMD) to be calculated using the equation $((\text{DM intake} - \text{faecal DM}) / \text{DM intake}) * 100\%$.

3.5.6 Urine samples

Urine samples were collected on the 18 October, 1 November, 8 November, 15 November, 22 November and 25 November 2010 in 70 ml plastic containers. Urine was collected by stimulating the cow to urinate by massaging below the vulva. Samples were collected mid-stream and were immediately treated with 10 drops of sulphuric acid. Samples were refrigerated at 4° C, before being sub-sampled into 2 ml plastic vials and frozen. These samples were later analysed by Lincoln University (LU) Analytical Services to determine: kinetic UV assay using Roche Urea/ BUN Kit (Roche Cobas Mira Plus CC) for urea; combustion under oxygen supply and high temperatures using Variomax CN Analyser; Elementar for total N; Enzymatic UV Method using Randox Ammonia Kit (Roche Cobas Mira Plus CC) for ammonia; and Kinetic Colorimetric Assay using Roche Creatinine Jaffe Kit for creatinine (Roche Cobas Mira Plus CC). Urinary nitrogen was calculated using an

equation developed by Pacheco *et al.* (2009), where total urine collection was performed in lactating pasture-fed dairy cows (n=15): UN (urinary nitrogen) (g/d) = ((21.9 * BW)/creatinine (mg/kg))*N (g/kg).

3.5.7 Blood samples

Blood samples were obtained from the coccygeal vein of all of the cows twice daily (0800 h and 1600 h) on the 18 October, 1 November, 8 November, 15 November, 22 November and 25 November 2010. Blood (10 ml) was collected from the coccygeal vein into EDTA coated tubes (15% tripotassium EDTA; Vacuette, Greiner Bio-one). Blood tubes were immediately placed on ice and taken to the LU lab, where plasma was harvested by spinning at 3000 x g for 15 m at 4° C (Mistral 3000, England). Plasma was pipetted into one 5 ml plug-top test tube (Biolab, Christchurch, New Zealand) and this was labelled and stored at -20° C before being measured in the LU lab by: Enzymatic Assay using the Randox NEFA Kit (Randox RX Daytona) for NEFA; Kinetic Enzymatic Method using the Randox BHB Kit (Randox RX Daytona) for β -OHB; Enzymatic Kinetic UV assay using Randox Kinetic Kit (Randox RX Daytona) for urea; Kinetic Colorimetric Assay using the Randox Creatinine Kit (Randox RX Daytona) for creatinine and Enzymatic Hexokinase Assay using the Randox Glucose Kit (Randox RX Daytona) for glucose.

3.5.8 Trace minerals and vitamins

Trace minerals and vitamins were not measured in this trial as they were assumed to be within adequate dietary values for all of the cows. During the trial three cows showed symptoms of magnesium deficiency-induced staggers, brought on at oestrus. The cows displayed physical symptoms of inappetance, staggy gait, malaise and excessive teeth-grinding. These cows were administered with 500 ml of a magnesium glucose solution (Glucalmag) intravenously immediately on presentation of these symptoms and their behaviour was monitored more closely over the next 24 h. All three cows resumed normal behaviour within 1 h of treatment and neither milk production nor feeding behaviour was seen to be adversely affected.

3.5.9 Statistical analysis

For each cow, the mean of the six treatment days (18 October, 1 November, 8 November, 15 November, 22 November and 25 November 2010) was calculated and then analysed in two stages. In the first stage, all of the treatments were included in a one-way ANOVA model in GenStat, using cow as a replicate. Where treatment effects were significant a least significant difference (LSD) test was used to test differences among treatment means. Following this, a

separate analysis using the four supplemented treatments was conducted. Data were analysed by ANOVA of a 2 x 2 factorial model: $y_{ijk} = \mu + A_i + B_j + A_i * B_j + e_{ijk}$. Where: y_{ijk} = k^{th} observation in the i^{th} diet treatment group A and j^{th} timing treatment group B; μ = general mean; A_i = fixed effect of the i^{th} diet treatment group; B_j = fixed effect of the j^{th} timing treatment group; $A_i * B_j$ = fixed interaction between treatment groups and e_{ijk} = random residual error.

Apparent DMI (calculated from pre and post grazing values), pre- and post- grazing pasture mass, botanical composition, blood, urine, faeces and body weight measurements were analysed by one way ANOVA (5 levels), in a one-stage process, using measurement day as a replicate. Differences were considered statistically significant at $P \leq 0.05$.

Chapter 4 Results

4.1 Pasture

4.1.1 Pasture mass, botanical composition and nutritive value

Average pre-grazing pasture mass ranged from 2450 kg DM/ ha to 2705 kg DM/ ha and post-grazing pasture masses averaged 1449 kg DM/ ha. There were no significant differences between treatments for pre- or post- grazing pasture masses, botanical composition or nutritive value of the pasture.

Table 3 Mean pre- and post- grazing pasture masses; botanical composition and nutritive value of the of the pasture consumed by each of the treatment groups. For treatment codes, refer below (SEM = pooled standard error of the mean)

Component	Treatment					SEM	P value ¹
	C	E9	E1	P9	P1		
Pre-grazing pasture mass, kg DM/ ha	2589.0	2577.7	2449.3	2704.9	2692.9	52.5	NS
Post-grazing pasture mass, kg DM/ ha	1578.3	1454.5	1330.4	1507.6	1372.3	33.2	NS
Botanical composition							
<i>Vegetative ryegrass, %</i>	56.0	58.0	53.5	55.5	54.0	0.8	NS
<i>Reproductive ryegrass, %</i>	33.9	31.0	34.6	32.8	35.3	0.8	NS
<i>White clover, %</i>	7.9	8.5	9.0	8.5	8.0	0.2	NS
<i>Weeds, %²</i>	0.7	0.8	0.9	0.5	0.6	0.1	NS
<i>Dead material, %</i>	1.5	1.7	2.0	2.7	2.1	0.2	NS
Nutritive value							
<i>DM, %</i>	229	229	234	231	227		
<i>NDF, g/ kg DM</i>	384	373	400	387	400		
<i>ADF, g/ kg DM</i>	215	209	222	219	221		
<i>WSC, g/ kg DM</i>	204	191	195	199	199		
<i>CP, g/ kg DM</i>	138	158	144	137	134		
<i>RDP, % CP³</i>	91.0	86.5	85.5	87.5	88.0		
<i>RUP, % CP³</i>	9.0	13.5	14.5	12.5	12.0		
<i>Lys, % EAA⁴</i>	10.5	10.5	10.5	10.5	10.5		
<i>Met, % EAA⁴</i>	3.9	3.9	3.9	3.9	3.9		
<i>DOMD</i>	79.2	79.7	78.4	78.9	78.8		
<i>MJME</i>	12.5	12.3	12.2	12.2	12.2		

¹ Means in the same row with the same superscript do not differ significantly, according to LSD test following a significant ANOVA result (P<0.05); ² Predominant weed observed in the sward was Annual poa (*Poaceae*); ³ Neutral detergent- resistant N; ⁴ Estimated from NRC (2001); WSC- water soluble carbohydrates; DOMD- degradable organic matter digestibility; C: 17.0 kg DM pasture/c/d; E9: energy concentrate allocated 9 h before pasture; E1: energy concentrate allocated 1 h before pasture; P9: RUP concentrate allocated 9 h before pasture; P1: RUP concentrate allocated 1 h before pasture

4.2 Total diet nutritive composition

The composition of the diets in the five treatments is shown below in Table 4. The pasture intakes of the treatments were estimated from pre- and post- grazing residuals using a RPM and averaged for each treatment and the mean concentrate intake per treatment group over the experiment was used. Total dry matter intake (pasture + supplement) was lowest in the E1 group (11.3 kg) and highest in the P9 group (14.1 kg), with the other groups averaging 13.5 kg. The average pasture intake of the supplemented groups was 10.0 kg, with the P9 group consuming the least (9.4 kg) and the E9 group consuming the most (10.5 kg). Supplement intake was lowest in the E1 group (1.4 kg) and highest in the P9 group (4.7 kg), with the other two groups averaging 3.1 kg. There was only minor substitution of pasture occurring in two of the supplemented groups, which included the E9 and P9 groups (0.1 kg of pasture per kg of supplement). The NDF intakes of the E groups were lowest at 4.3 kg. The CP intake of the control group was lowest at 1.8 kg and highest in the P9 group at 2.5 kg. Total energy intake was lowest in the E1 group at 140.3 MJ and highest in the P9 group at 182.8 MJ. The other three groups averaged 170.1 MJME/ d.

Table 4 Nutritional composition of treatment diets of cows fed pasture only, or supplemented with wheat and canola

Component	Treatment ¹					
	C	E9	E1	P9	P1	SD
DMI, kg/d	13.3	13.6	11.3	14.1	13.4	1.1
DM, %	22.9	36.3	30.9	43.2	36.4	7.6
<i>Total, % BW</i>	3.1	3.1	2.4	3.1	2.9	0.3
Pasture, kg/d	13.3	10.5	9.9	9.4	10.3	1.5
Supplement, kg/d	0.0	3.1	1.4	4.7	3.1	1.8
NDF, % DM	38.4	31.7	37.9	32.4	35.1	3.1
NDF intake, kg/d	5.1	4.3	4.3	4.6	4.7	0.3
<i>Total, % BW</i>	1.1	1.0	0.9	1.0	1.0	0.1
ADF, % DM	21.5	17.2	21.6	19.1	20.2	1.8
ADF intake, kg/d	2.9	2.3	2.4	2.7	2.7	0.2
<i>Total, % BW</i>	0.6	0.5	0.5	0.6	0.6	0.1
CP, % DM	13.8	15.1	16.6	17.8	16.2	1.5
CP intake, kg/d	1.8	2.1	1.9	2.5	2.2	0.3
<i>Total, % BW</i>	0.4	0.5	0.4	0.6	0.5	0.1
N intake, g/d	293.7	328.6	300.1	401.6	347.3	43.4
RDP, % CP	91.0	80.4	83.2	80.0	82.8	4.4
RDP, kg/d	1.6	1.7	1.6	2.0	1.8	0.2
RUP, % CP	9.0	19.6	16.8	20.0	17.2	4.4
RUP, kg/d	0.2	0.4	0.3	0.5	0.4	0.1
Lys, % EAA ²	10.5	9.7	10.8	10.6	10.6	0.4
Met, % EAA ²	3.9	4.2	4.0	4.1	4.1	0.1
Pasture, MJ	166.3	128.1	120.8	114.7	125.7	20.3
Supplement, MJ	0.0	45.0	19.5	68.1	45.3	26.3
<i>Total, MJ³</i>	166.3	173.1	140.3	182.8	171.0	15.9
DOMD	79.2	83.9	79.0	81.9	80.9	2.0
NE _L intake, MJ/d	109.1	115.9	83.1	125.6	113.8	15.9
NE _L intake, l/d	16.5	17.6	12.6	19.0	17.2	2.4

¹ C: control, E9: wheat supplement 9 h before pasture, E1: wheat supplement 1 h before pasture, P1: wheat and canola supplement 1 h before pasture, P9: wheat and canola supplement 9 h before pasture); ADF- acid detergent fibre; NE_L- net energy lactation; MJ- megajoules of ME; ² Estimated from NRC (2001); ³ Diets designed to be iso-energetic, but due to feeding behaviour of cows this was not seen to occur

4.3 Milk production and composition

The effect of the treatments on milk parameters is shown in Table 5. The control and E9 groups had significantly lower milk yields than the other treatment groups (21.8 l, compared to 25.4 l). The control and 9 h treatments had significantly higher percentages of protein in their milk (mean: 3.82%), than the 1 h treatments (mean: 3.7%). The control and E9 groups had significantly lower yields of protein (mean: 0.85 kg), compared to the other treatments (mean: 0.93 kg). There were no significant differences between the treatment groups for milk fat percentage. The P groups had significantly higher MUN concentrations than the E treatments (3.2 and 2.5 mmol/l for the P and E groups, respectively). The control treatment produced significantly less milk fat (1.16 kg/ d) than the mean of the other treatments (1.18 kg/ d). The E9 group produced significantly less MS than the other supplemented treatments (1.86 kg compared to >1.99 kg). The E1 and P treatment groups had the highest yields of MS, averaging 2.17 kg/d. As a percentage of N intakes, milk N was highest in the E1 group (49.6%) and lowest in the E9 group (40.9%), with the other groups averaging 40.3%. Analysis of the supplemented groups only showed a significant interaction for milk yield, protein yield, fat yield and MS yield. In the wheat-only groups, these response variables were lower when the supplement was fed 9 h, rather than 1 h, but in the P groups there was no effect on time of feeding supplement.

Table 5 Mean milk parameters of cows fed treatment diets of pasture only, or supplemented with wheat and canola

Parameter	Treatment ¹					SEM	P<0.05 ²			
	C	E9	E1	P9	P1		Trt	D	t	d*t
Milk yield, l/d	21.7 ^a	21.9 ^a	25.0 ^b	25.5 ^b	25.6 ^b	0.41	<0.001	<0.001	NS	0.001
Protein, %	3.87 ^a	3.88 ^a	3.69 ^b	3.80 ^a	3.70 ^b	0.07	0.014	NS	0.002	NS
Protein, kg/d	0.85 ^a	0.84 ^a	0.93 ^b	0.95 ^b	0.90 ^b	0.03	<0.001	NS	0.012	<0.001
Fat, %	5.60	5.12	5.20	5.15	5.25	0.18	NS	NS	NS	NS
Fat, kg/d	1.16 ^a	1.08 ^b	1.28 ^c	1.24 ^{ac}	1.24 ^{ac}	0.05	<0.001	0.046	0.011	0.007
MS, kg/d	1.99 ^a	1.86 ^b	2.16 ^c	2.22 ^c	2.14 ^c	0.07	<0.001	<0.001	NS	<0.001
Milk N, g/d	136.0 ^a	134.4 ^a	148.8 ^b	152.0 ^b	144.0 ^b	4.8	<0.001	NS	NS	NS
N Milk/ N intake, %	46.3	40.9	49.6	37.8	41.5					
MUN, mmol/l	2.91 ^a	2.50 ^b	2.52 ^b	3.29 ^c	3.04 ^{ac}	0.26	0.009	<0.001	NS	NS

^{a, b, c} Means in the same row with the same superscript do not differ significantly, according to LSD test following a significant ANOVA result (P<0.05); ¹ C: control, E9: wheat supplement 9 h before pasture, E1: wheat supplement 1 h before pasture, P1: wheat and canola supplement 1 h before pasture, P9: wheat and canola supplement 9 h before pasture; ² Trt: treatment, D: diet, t: timing, d*t: diet/ timing interaction for factorial analysis of supplemented groups only

4.4 Body weight and body condition score

The effect of the treatments on body weight and BCS is shown in Table 6. On average the cows gained 10.5 kg/ cow and lost 0.2 of a point unit of a BCS. However, there were no significant treatment effects on change in BW or BCS over the experimental period.

Table 6 Change in body weight (BW) and body condition score (BCS) of cows in treatment groups fed pasture only, or supplemented with wheat and canola

Parameter	Treatment ¹					SEM	P<0.05 ²			
	C	E9	E1	P9	P1		Trt	D	t	d*t
Change BW, kg	3.5	14.7	12.9	21.6	-0.4	8.7	NS	NS	NS	NS
Change BCS ³	-0.41	0.09	-0.23	-0.41	-0.24	0.33	NS	NS	NS	NS

a, b, Means in the same row with the same superscript do not differ significantly, according to LSD test following a significant ANOVA result (P<0.05); ¹ C: control, E9: wheat supplement 9 h before pasture, E1: wheat supplement 1 h before pasture, P1: wheat and canola supplement 1 h before pasture, P9: wheat and canola supplement 9 h before pasture; ² Trt: treatment, d: diet, t: timing, d*t: diet/ timing interaction for factorial analysis of supplemented groups only; ³ 6-point system where 1 is thin and 6 is obese

4.5 Urine

The effect of the treatments on urine parameters are shown below in Table 7. The E1 treatment group had a significantly lower concentration of urea N than the other supplemented treatment groups (95.3 mmol/l, compared to >105.3 mmol/l). The E groups had significantly lower urea N concentrations (101 and 130 mmol/l in the E and P groups, respectively). The total amount of N excreted in the urine was lower in the E groups (157 g/d) than the P groups (177 g/d). There were no significant differences between the control group and any of the supplemented treatments for ammonia or creatinine concentration in the urine. Analysis of the supplemented groups only showed a significant effect of diet for urea concentrations. Analysis of supplemented groups only showed urine N concentration to be significantly greater in the P9 group, compared to the E groups (0.74% and 0.63% in the P9 and E groups, respectively). Urine N excretion was greater in P groups (177.3 g/d) than E groups (156.8 g/d) but there was no significant effect of timing. As a percentage of N intake, urine N was highest in the control group (62.1%), with the remaining four groups having very similar urine N percentages, with an average of 48.7%.

Table 7 Mean urine measurements of cows in treatment groups fed pasture only, or supplemented with wheat and canola

Urine	Treatment ¹					SEM	P<0.05 ²			
	C	E9	E1	P9	P1		Trt	d	t	d*t
Urea N, mmol/l	105.3 ^a	106.7 ^a	95.3 ^b	130.8 ^c	129.3 ^c	13.71	0.039	0.005	NS	NS
NH ₃ , mmol/l	1.18	1.49	1.66	1.49	2.30	0.49	NS	NS	NS	NS
Creatinine, mmol/l	2.92	3.61	3.32	3.37	3.32	0.34	NS	NS	NS	NS
Urine N conc., %	0.73	0.62	0.63	0.74	0.68	0.05	NS	<0.001	NS	NS
N excretion, g/d	182.3	156.0	157.5	184.1	170.4	14.1	NS	0.038	NS	NS
N urine/ N intake, % ³	62.1	47.5	52.5	45.8	49.1					

^{a, b}, Means in the same row with the same superscript do not differ significantly, according to LSD test following a significant ANOVA result (P<0.05); ¹ C: control, E9: wheat supplement 9 h before pasture, E1: wheat supplement 1 h before pasture, P1: wheat and canola supplement 1 h before pasture, P9: wheat and canola supplement 9 h before pasture; ² Trt: treatment, d: diet, t: timing, d*t: diet/ timing interaction for factorial analysis of supplemented groups only; ³ Calculated from other variables

4.6 Faeces

The effect of the treatments on faecal parameters is shown in Table 8. The DOMD percentage was lowest in the control group (79.1%) and highest in the E9 group (83.2%). There were no significant differences between any of the treatments for faecal DM percentage (mean: 12.0%). The IVOMD percentages were similar for all of the treatments (mean: 88.1%), as were the non-digestible DM amounts (mean: 2.5 kg/d). Analysis based on supplemented groups only showed a significant treatment effect of diet on faecal N excretion, with the P groups excreting significantly more N (20.6 g/d) than the E groups. The proportion of N in faeces of N intake was lowest in the P9 group (22.8%) and highest for the control group (34.1%). The other three treatment groups averaged 26.9%.

Table 8 Mean faecal measurements of cows in treatment groups fed pasture only, or supplemented with wheat and canola

Parameter	Treatment ¹					SEM	P<0.05 ²			
	C	E9	E1	P9	P1		Trt	D	t	d*t
DOMD, %	79.1	83.2	80.4	81.8	81.0	0.69				
Faecal DM, %	11.85	11.61	11.59	12.41	12.34	1.26	NS	NS	NS	NS
IVOMD, % ³	88.2	88.4	88.4	87.6	87.7	0.17				
Non-digestible DM, kg/d	2.78	2.35	2.32	2.62	2.59	0.09				
Faecal N, % DM	3.60	3.60	3.59	3.49	3.64	0.09	NS	NS	NS	NS
Faecal N, g/d	103.5	81.7	83.0	96.0	97.2	2.36	NS	<0.001	NS	NS
N faeces/ N intake, %	35.2	24.9	27.7	23.9	28.0					

^{a, b, c} Means in the same row with the same superscript do not differ significantly, according to LSD test following a significant ANOVA result (P<0.05); ¹ C: control, E9: wheat supplement 9 h before pasture, E1: wheat supplement 1 h before pasture, P1: wheat and canola supplement 1 h before pasture, P9: wheat and canola supplement 9 h before pasture; ² Trt: treatment, d: diet, t: timing, d*t: diet/ timing interaction for factorial analysis of supplemented groups only; ³IVODM=(DMI–faecal DM)/DMI*100

4.7 Blood

The effect of the treatments on blood parameters is shown in Table 9. The control group had a significantly lower concentration of NEFA in the blood (0.24 mmol/l) than the other treatment groups. The concentration of β -OHB in the blood was found to be similar for all of the treatment groups. The E1 treatment group had a significantly lower urea N concentration (2.9 mmol/l) than the other treatments. Following this, the control and E9 treatments had significantly lower concentrations of urea N (mean: 3.22 mmol/l) than the P treatment groups (mean: 3.9 mmol/l). There were no significant differences between any of the treatment groups for the concentration of creatinine in the blood. The P9 group had a significantly higher concentration of glucose in the blood (3.8 mmol/l), compared to the other four treatments (mean: 3.7 mmol/l). Analysis based on the supplemented groups only showed that there was a significant effect of timing on the concentrations of NEFA and urea N in the blood. The 9 h treatment groups had significantly lower concentrations of NEFA and significantly higher concentrations of urea N than the 1 h treatments. Analysis based on supplemented groups only showed no significant interactions between diet and timing for any parameter.

Table 9 Mean blood measurements of cows in treatment groups fed pasture only, or supplemented with wheat and canola

Parameter	Treatment ¹					SEM	P<0.05 ²			
	C	E9	E1	P9	P1		Trt	d	t	d*t
NEFA, mmol/l	0.24 ^a	0.32 ^b	0.50 ^c	0.32 ^b	0.50 ^c	0.07	0.005	NS	<0.001	NS
β -OHB, mmol/l	0.62	0.74	0.65	0.61	0.61	0.06	NS	NS	NS	NS
Urea N, mmol/l	3.24 ^a	3.20 ^a	2.94 ^b	4.01 ^c	3.74 ^c	0.33	0.008	NS	<0.001	NS
Creatinine, mmol/l	0.065	0.066	0.065	0.063	0.065	0.00	NS	NS	NS	NS
Glucose, mmol/l	3.66 ^a	3.71 ^a	3.66 ^a	3.79 ^b	3.68 ^a	0.05	NS	0.037	NS	NS

^{a, b}, Means in the same row with the same superscript do not differ significantly, according to LSD test following a significant ANOVA result (P<0.05); ¹ C: control, E9: wheat supplement 9 h before pasture, E1: wheat supplement 1 h before pasture, P1: wheat and canola supplement 1 h before pasture, P9: wheat and canola supplement 9 h before pasture; ² Trt: treatment, d: diet, t: timing, d*t: diet/ timing interaction for factorial analysis of supplemented groups only

Chapter 5 Discussion

The experiment was designed to test the effect of feeding energy and high-RUP supplements at different times, relative to a major pasture meal, on MS production and N utilisation. This was achieved by comparison of cows offered wheat (energy) and canola meal (RUP) in the morning and afternoon prior to their evening allocation of pasture and also by comparison to a pasture-only group. Diets were designed to be iso-energetic and iso-nitrogenous. However, cows not meeting target supplement or pasture DM intakes, the only diets that were iso-energetic were the control, E9 and P1 diets (mean: 170.0 MJ/d). The diets that were iso-nitrogenous were the control and E1 groups (mean: 297 g/d) and the E9 and P1 groups (mean: 338 g/d). Measurements of pasture, milk, urine and faeces parameters in response to treatments, gave the following key results:

1. Average milk solid yield in the P groups was significantly higher than the average of the C group (2.18 kg/d compared to 1.99 kg/d).
2. Urinary and faecal N losses (as a percentage of N intakes) in the E groups were lower than those of the C group (12.1% and 7.4% reduction in urinary and faecal losses, respectively).
3. Urinary and faecal N losses (as a percentage of N intakes) in the P groups were higher than the E groups (2.6% and 1.8% increases in urinary and faecal losses, respectively), but were unaffected by timing of supplementation.
4. Feeding the supplement 9 h before pasture reduced the mean MS production from 2.16 kg MS/d (1 h groups) to 1.86 kg MS/d in the E groups, but not in the P groups.

RUP Supplementation Effect on Milk Yield

Milk solid production was greater in RUP treatments than the control at both timings of supplementation. In contrast, milk solid production in the E treatments was greater than the control in the E1 treatment only. This result contrasts with the findings of Penno and Carruthers (1995), who found no increase in milk production when 300 g/d of fish meal was supplemented to pasture-fed cows, but is in agreement with other studies. Bargo *et al.* (2003) in a review of eight studies reported a mean increase of 0.8 kg MS/d for each 100 g/d RUP (sources included: fishmeal, blood meal, feather meal and heat-treated rapeseed meal) in an

iso-energetic diet. The majority of the studies were implementing a total mixed ration diet, as opposed to a pasture-based diet.

The RUP supplemented diets had higher intakes of CP than either the C or E groups (1.8, 2.0 and 2.4 kg/d in the C, E and P groups, respectively). As high yielding dairy cows require at least 17.0% CP from DM to optimise milk production this would have meant that the C and E supplemented cows were consuming diets deficient in CP (mean: 15.2% CP) (Hongerholt & Muller, 1998). In contrast, the P cows were consuming diets which had a CP percentage of 17.0%. The low N content in the diet could have negatively affected the level of milk production achieved by the C and E groups. However, N percentage in milk as a percentage of N intake across the treatments was higher than the range value other researchers have found (43% in this study, compared to 22 – 27% in other studies) and indicates that the pasture consumption of some treatments may have been higher than those recorded (Pacheco *et al.*, 2009, Tas *et al.*, 2005, Vibart *et al.*, 2009). Therefore, underestimations of pasture intake may have underestimated CP intake and the treatments may have been consuming diets adequate in CP for the level of milk production achieved.

The pasture was low in CP (mean: 14.2%) and the reason for this may have been due to the reproductive stage of the herbage, as well its low legume content. The low CP content of the pasture occurred despite the fact that the pasture was fertilised with 50 kg N/ ha four weeks before the experiment started. At the commencement of the trial, the pasture sward was predominantly made up of vegetative material (58%), with only a small proportion of reproductive matter (9%). However, over November the predominant ryegrass species in the sward became reproductive and by the end of November the percentage of reproductive material was higher than the vegetative material (56%, compared to 41%).

The response of the RUP supplemented groups may also have been a consequence of the extra energy these groups consumed (mean increase: 20.5 MJ/c/d). Based on a requirement of 77 MJ/ kg MS for a cross-bred cow, this would be equivalent to an extra 0.27 MS/d for the RUP supplemented groups (0.17 kg more MS/d was produced by RUP supplemented cows) (DairyNZ, 2010). When the increased energy is taken into account the observed responses is less than expected. Although the diets were designed to be iso-energetic, differences in voluntary intake of pasture and supplement resulted in some groups consuming more energy than others. The E9 and P1 diets were iso-energetic with each other and these groups

consumed 170.0 MJ/d. This compares to the E1 and P1 groups consuming 140 and 183 MJ/d, respectively.

The optimum amounts of Lys and Met as percentages of total EAA have been stated to be 16% and 5% by Schwab (1994). In this experiment, the treatment groups had mean Lys and Met percentages of EAA of 10.5% and 4%. This was less than optimal for protein production in milk. Although some of the treatment groups consumed more total CP, all of the diets had similar proportions of the two limiting EAA, so the significance of this was reduced.

Energy Supplementation Effect on Milk Yield

In the groups just supplemented with wheat, milk yield and MS yield in the E1 treatment group (2.16 kg/cow/d) was greater than the control (1.99 kg/cow/d), but was lower than the control in E9 group (1.86 kg/cow/d). This was a surprising result as total DMI was lower in the E1 group than the E9 group (13.6 kg/d in the E9 group, compared to 11.3 kg/d in E1 group, reflecting a reduced DM intake of wheat). This equated to a difference of 28.1 MJ, which is equivalent to 0.36 kg MS/d (DairyNZ, 2010). In terms of BCS change over the experimental period, there was a difference (statistically insignificant) observed between the E9 and E1 groups (0.09 of a BCS point gain in the E9 group and 0.23 of a BCS point loss in the E1 group). If this difference in BCS was measured in energy terms (26 kg/ BCS for a 400 kg cross-bred cow * 50 MJME/ kg LWT gain or 37 MJME/ kg LWT loss) the E1 group would have had 10.3 MJ/d more energy available for MS production than the E9 group (DairyNZ, 2010). This equates to the E1 group being able to produce an extra 0.13 MS/d. The difference in milk production between the E9 and E1 groups was 0.13 MS/d. Therefore it can be surmised that the difference in BCS accounts for the differences in milk production between these groups.

It is not clear why wheat intake was markedly lower in the E1 group, compared to the E9 group (1.4 and 3.1 kg/ d in the E1 and E9 groups, respectively). The E9 and E1 cows were going into similar pre-grazing pasture masses (2550 kg DM/ha and 2450 kg DM/ha for the E9 and E1 groups, respectively) and both groups were removing around 1100 kg DM/ha, leading to similar pasture DM intakes. This indicates that there was no difference in substitution of pasture for wheat between the groups. It is plausible that for the E1 group, allocation of their supplement close to the time when pasture was allocated resulted in less wheat being

consumed because of an expectation of pasture allocation. Alternatively, processes related to digestion of forage may have created conditions that reduced supplement DM intake.

Energy Supplementation Effect on Nitrogen Utilisation

There was a reduction in urinary and faecal N losses (as a percentage of N intake and total N excreted) in the E groups, compared to the control (12.1% and 7.4% reduction in urinary and faecal losses, respectively). A reduction in N losses when a RFCHO source is fed is similar to what others have found, including Schroeder *et al.* (2004). Schroeder *et al.* (2004) stated that the composition of pasture with its relative excess of N to carbon precursors can lead to reduced microbial protein (MicP) synthesis in grazing cows. By providing the rumen microbes with a source of energy when rumen ammonia concentrations are high, the rumen microbes can better utilise the N in the urine and less will be detoxified and excreted. This is an important finding as it provides a method to dilute the N concentration of the diet as well as promoting higher levels of milk production.

The chemical composition of the RFCHO source will also play a large part in determining its effect on the nitrogen utilisation of the animal. A slow release RFCHO source, such as maize silage, will have a more prolonged effect on MicP synthesis than a fast release RFCHO source, such as wheat. This will be relevant to the grazing behaviour of the cows, whether the bulk of pasture intake takes place over a number of hours, or in one shorter bout spanning between one and three hours. Matching a slow release RFCHO source, like maize silage, with cows grazing pasture over a number of hours should have a more beneficial effect on nitrogen utilisation than supplementing the grazing cow's diet with wheat. Similarly, feeding wheat to cows eating the bulk of their pasture in less than three hours would potentially increase nitrogen utilisation more than would feeding a slow release RFCHO source.

RUP Supplementation Effect on Nitrogen Utilisation

Feeding the high-RUP supplement reduced urinary and faecal N losses, compared to the control group. However, the P groups had increased urinary and faecal N losses, compared to the E groups. High-RUP supplementation also increased urine urea N from that of the control and the E groups significantly. The urine urea N concentration of the control and E groups were 105 and 101 mmol/l, respectively. This compares to 130 mmol/l in the P groups. There

was also a significant effect on MUN concentrations between the E and P treatment groups, with the E treatments having an average MUN concentration 26% lower than the P treatments. This was similar to the differences seen in BUN, with the P treatments having BUN concentrations 26% higher than the E treatments. As urea diffuses into and out of the mammary gland and equilibrates with the concentration of urea in the blood, it is expected that the concentration of MUN will be proportional to BUN (Jonker *et al.*, 1998). These findings refute the hypothesis, which predicted N losses to be the same in both the E and P treatments. The reasons for this are probably due to the higher N intakes of the P groups (66 g/d increase) and the surplus AA in the P rations, which may have entered the mammary gland before being metabolised, when the supply of EAA was limiting for milk production (Lobley and Lapierre, 2001). The higher MUN and BUN concentrations of the P treatments indicate lower N utilisation efficiency from these groups.

Effect of Timing on Milk Yield

The 1 h groups produced a mean 0.11 kg MS/d more than the 9 h groups (2.15 kg MS/d, compared to 2.04 kg MS/d). This finding was not statistically significant however. This finding occurred despite the fact that the 9 h groups consumed 23 MJME/d more than the 1 h groups (equivalent to 0.29 kg MS/d) (DairyNZ, 2010). It is thought that the 9 h groups would have been in a more positive energy balance as the percentage of protein in the milk was higher (mean: 3.8%), compared to 3.7% in the 1 h groups. A higher protein percentage in the milk is indicative of a more positive energy balance (Bargo *et al.*, 2002, Delaby *et al.*, 2001). A comparison of the net energy balance of the groups with their predicted net energy balances (based on milk production) highlighted the fact that the 1 h groups were putting 85.2 MJ/d more into milk production than could be accounted for based on their energy consumption. The differences (non-significant) in liveweight mobilisation do not help to explain the higher than expected milk production of the 1 h group, as the 1 h groups would have only had an extra 0.03 kg MS/d at their disposal for milk production if BCS changes are accounted for (DairyNZ, 2010). Furthermore, it cannot be surmised that this difference was due to the improved rumen nitrogen utilisation in the 1 h groups, as the excretion of N in the urinary and faecal fractions as a percentage of N intake in the 1 h groups was higher than in the 9 h groups. The only possible explanation that can be offered is that some of the cows in the 1 h groups had better feed conversion efficiency than some of those in the 9 h group.

There was no significant effect of timing on urinary and faecal N losses. The excretion of N as a percentage of N intake was reduced by 4.1% and 3.2% in the urinary and faecal fractions of the 9 h groups, compared to the 1 h groups. However, this result was non-significant. Despite being insignificant, this finding agrees with previous studies showing an effect of the timing of supplementation, including a study by Mitani *et al.* (2005) and a study by Gregorini *et al.* (2010). Mitani *et al.* (2005) observed greater milk N output in cows fed a maize silage supplement pre-grazing, compared to those fed the same supplement post-grazing. Gregorini *et al.* (2010) found effluent NH₃ % of total N flow to be reduced from 31.4% to 22.7%, when the RFCHO source was given 9 h before pasture intake, compared to 1 h before. This equated to a 28% reduction in NH₃ % of total N flow, compared to when the high-RFCHO was fed 1 h before pasture intake. The reason for this response was probably due to the supply of RFCHO in the rumen being more closely matched to the concentration of ammonia in the rumen. The reason for differences between this study and others is unclear but may reflect the type of supplement used (wheat versus maize silage), which may drive different pathways of rumination and energy availability. Further, the Gregorini and Soder (2009) study utilised a continuous culture fermentor, which may not accurately reflect the pasture consumption pattern of dairy cows over a 24 h grazing allocation.

5.1 BW Changes

There were no statistical differences observed between any of the treatments for either BW or BCS measurements. The mean BW gain over the experimental period was 10.5 kg and the mean BCS change was a 0.2 BCS loss. Despite the fact that the diets were designed to be iso-energetic it was theorised that the supplemented groups would gain more weight (and body condition) over the experimental period due to the composition of the supplemented diets being higher in starch. This may not have occurred in the E1 group owing to their reduced energy intakes and high level of milk production achieved. Notwithstanding the fact that the BCS gain changes observed between the E9 and control groups were statistically insignificant, it could be noted that the E9 group was the only treatment group to gain in BCS during the experiment (0.09 BCS increase). The reason behind the P groups losing condition during the experiment could have been due to the increased supply of EAA entering the mammary gland that may have increased LWT mobilisation to support milk production.

5.2 Economics

In the short term, milk responses determine whether a supplementation strategy is profitable or not, depending on milk and concentrate prices (Bargo *et al.*, 2003). The cost of RFCHO and RUP supplementation must therefore be considered as the use of supplements is only economical if the value of that additional milk exceeds the supplement cost. If the milk to feed ratio approaches one or less, then the feeding of the supplement becomes unprofitable, with the possible exception of early lactation, high genetic merit cows.

The cost of wheat grain (\$310/T; contract) and the cost of canola meal (\$620/T; contract) must be weighed up against the extra profit generated in MS from their use. As shown in Table 10, the E9, E1, P9 and P1 treatment groups produced a mean -0.13, 0.17, 0.23 and 0.15 kg MS each day, respectively. At the current milk price (\$6.75/kg MS) this would have caused a daily loss of \$2.46, \$0.43, \$0.96 and \$1.50 per day for the E9, E1, P9 and P1 treatments, respectively. It must be noted that this simplistic cost analysis does not include any of the longer term indirect effects of supplementation, such as increased/ decreased reproductive efficiency, increased/decreased body condition or increased labour and infrastructure inputs.

Table 10 Short- term economic viability of E and P treatment groups

Parameter	E9	E1	P9	P1
Cost, \$/c/d	\$1.58	\$1.58	\$2.51	\$2.51
Additional MS ¹ produced, kg/d	-0.13	0.17	0.23	0.15
Profit for MS, \$/c/d	-\$0.88	\$1.15	\$1.55	\$1.01
Net profit/loss, \$/c/d	-\$2.46	-\$0.43	-\$0.96	-\$1.50

¹ MS- milk solids (kg/d)

Chapter 6 Conclusion

6.1 Research contribution

6.1.1 RFCHO supplementation

It is already known in the dairy industry that under certain MS prices and supplement costs, providing a proportion of the cow's diet as a high-RFCHO, low-NDF supplement can be economically-advantageous due to the extra MS produced. This is because the cow has a physical limit to the amount of DM/NDF she can consume and these high energy supplements allow higher energy inputs without the associated DM input that comes with grass consumption. However, in this experiment the treatment groups provided with a high-RFCHO supplement (E9) consumed approximately the same amount of energy as the pasture-fed control group. Therefore, as only the fractionation on RFCHO: CP was altered between these treatment groups it was unlikely that any significant increases in MS production or N utilisation would be seen, as was found to be the case.

6.1.2 High-RUP supplementation

In the NZ dairy industry, the provision of supplements full-stop is a contentious issue. Add to this the feeding of a supplement that is both high in protein (specifically relevant to a NZ context where the pasture on offer is excessive in CP) and high in cost (owing to its high-protein level) and you have a very heated debate in some dairy circles. Despite this, sometimes dairy industry professionals advocate the feeding of high-RUP supplements for their proposed advantage over other, high-CP feeds. In this experiment the high-RUP supplemented diet was found to significantly increase the level of milk production achieved. However, this increased level of production had a trade-off of a reduced efficiency of N utilisation and increased LWT mobilisation, which would have had environmental as well as financial implications. These would have to be taken into careful consideration when making the decision to supplement with a high-RUP feed.

6.1.3 Timing of feeding

Synchronising peak rumen ammonia concentrations with RFCHO supply may have a potential role in the dairy industry in the future when further research has been conducted. Potential benefits could include the use of energy supplements in wet and/or cold conditions

when cows are being stood-off pasture. In this experiment there was no significant effect on faecal and urinary N utilisation from providing the supplement 9 h pre-pasture, compared to 1 h pre-pasture.

6.2 Potential for further research

6.2.1 Reproductive effects

High levels of CP (>19%) in the diet have been associated with lower conception rates and early abortive losses in dairy cows (NRC, 1985). However, the effects of a moderate-CP diet (18%) with a high-RUP fraction are less well-known. This experiment took place during October when the cows had already started cycling activity and were being artificially inseminated as they came into oestrus. The high-RUP diet may have had an effect on the conception rates of these treatment groups and further research could potentially investigate the effects of a high-RUP diet on pregnancy rates in pasture-based systems.

6.2.2 Daily timing of supplementation

In this experiment, the supplement was provided as a single large allocation once per day and this resulted in wide perturbations of diet. However, what is commonly practised in the South Island is to feed supplements over two meals, usually during milking time in the milking parlour or on a feed-pad adjacent to the milking parlour. This makes sense as the cow has to consume less DM in one sitting and the flow of nutrients into the rumen is more regulated. This was not able to be replicated in this experiment due to economic constraints. There are potential advantages of dispersing the supplementation feeding between two feedings rather than one (as well as adjusting the relative amounts between the two feedings) and this could form the basis of a future research project.

6.2.3 Seasonal timing of supplementaton

Previous studies have documented time of day effects on pasture composition and DM intake, with increased DM intakes following evening allocation of pasture. The potential to target supplement allocation, relative to different times of the year is another opportunity for further research.

Chapter 7 References

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