Milk production and nitrogen partitioning in dairy cows grazing standard and high sugar perennial ryegrass with and without white clover, during spring and autumn

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ABSTRACT

Two field grazing experiments were conducted in New Zealand (NZ) in spring (Experiment 1; November 2008) and autumn (Experiment 2; April 2009) to evaluate the effects of feeding a high sugar perennial ryegrass (HSG; cv. AberDart; derived in the United Kingdom; UK) versus a NZ- derived control grass (cv. Impact) on milk production and estimated nitrogen (N) partitioning within the cow. Areas of both ryegrasses were replicated and sown with or without white clover (cl) (HSG+cl, control+cl, HSG and control). A cross-over design with four 10-day periods was used in each experiment, using 15 Friesian cows per treatment per period in Experiment 1 and 5 cows per treatment per period in Experiment 2. Treatment effects upon pasture botanical and chemical composition, cows' milk yield and composition, and estimated N partitioning were studied. Nitrogen partitioning was calculated using indirect methods.

Herbage concentrations of water soluble carbohydrates (WSC) were lower in autumn than in spring whilst crude protein (CP) concentrations were higher in autumn. Organic matter digestibility (OMD) and metabolisable energy (ME) concentration was similar in both seasons. There were no differences in the concentration of CP, WSC and dry matter (DM) among treatments in Experiment 1. The HSG+cl treatment had the lowest concentrations of neutral detergent fibre (NDF, 417 g/kg DM) and the highest content of ME (12.6 MJ/kg DM) and tended to have the lowest sward dead matter content compared with the other three treatments. In Experiment 2 both HSG treatments showed higher concentrations of WSC (15 g/kg DM) compared with the control, both with and without clover; the concentrations of NDF and acid detergent fibre (ADF) were the lowest for both HSG treatments.

In Experiment 1, cows grazing treatments with white clover produced more milk (1.6 kg/day) and more milk solids (MS; 0.16 kg/day) than cows grazing pure ryegrass swards ($P< 0.01$), with highest milk yields being from cows grazing the HSG+cl treatment (ryegrass cultivar x white clover interaction $P<0.05$). No differences in milk production were found in Experiment 2. Estimated urinary N excretion (g/day) was similar for all treatments in both seasons, although N intake differed among treatments. The proportion of N intake excreted in urine or secreted in milk was similar for all treatments in both experiments. Nitrogen output (g/day) in milk was the highest for the HSG+cl treatment in Experiment 1 but no differences were found in Experiment 2.

Data were combined from both experiments to study the effects of the herbage CP:WSC ratio upon estimated N partitioning between milk and urine. Mean ratios were 0.72 for spring herbage and 2.27 for autumn herbage. As the amount of WSC increased in the diet relative to

the amount of CP (thus a lower CP:WSC ratio) there was a significant increase in the amount of milk N secreted per unit of N intake in spring but not in autumn. The breakpoint in the relationship between the herbage CP:WSC ratio and the nitrogen utilisation efficiency for milk production (NUEm) was 1.32, and the NUEm for that breakpoint was 14 g milk N per 100 g N intake. Ratios below this point were associated with improved efficiency of converting pasture N to milk N; ratios above this point were not correlated with changes in N conversion efficiency.

It is concluded that the CP:WSC ratio in perennial ryegrass may be important in the partition of absorbed N into milk or urine. A NZ-selected HSG with a lower CP:WSC ratio is likely to have major benefits for pastoral farming in NZ. In order to be effective, a NZ-derived HSG should substantially increase WSC concentration in autumn pasture (from approximately 100 to 200 g/kg DM) whilst reducing CP content simultaneously (from 240 to 190 g/kg DM). The lower structural fibre and higher milk production for the HSG+cl treatment in both experiments suggest that under NZ conditions, best productive responses to HSG may be obtained in management systems that include white clover.

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AA	Amino acid(s)
ADF	Acid detergent fibre
ATP	Adenosine triphosphate
BCS	Body condition score
CHO	carbohydrate(s)
CP	Crude protein
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
FV	Feeding value
HSG	High sugar grass(es)
IBERS	Institute of Biological, Environmental and Rural Sciences
IGER	Institute of Grassland and Environmental Research
IRG	Italian ryegrass
LW	Liveweight
ME	Metabolisable energy
MJ	Mega joules
MP	Metabolisable protein
MPS	Microbial protein synthesis
MS	Milk solid(s)
MUN	Milk urea nitrogen
N	Nitrogen
NAN	Non- ammonia nitrogen
NDF	Neutral detergent fibre
NPN	Non protein nitrogen
NUEm	Nitrogen utilisation efficiency for milk production
NUEu	Nitrogen utilisation efficiency for urine excretion
NV	Nutritive value
NZ	New Zealand
OM	Organic matter
OMD	Organic matter digestibility
PKE	Palm kernel extract
RDP	Rumen degradable protein

TAA	Total amino acids
TMR	Total mixed ration
UDP	Undegradable protein
UK	United Kingdom
UN	Urinary nitrogen
VFA	Volatile fatty acid(s)
VFI	Voluntary feed intake
WSC	Water soluble carbohydrate(s)

CHAPTER 1

Literature Review

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Literature Review

1.1 Introduction

The dominant feature of New Zealand (NZ) dairy farming systems is that they are based on year round grazing of fresh temperate pasture plants. There is an extensive variety of forage types available to farmers. However, dairy production is largely based on grass-dominant pastures, mostly perennial ryegrass (*Lolium perenne*) combined with legumes (white clover-*Trifolium repens*) in a ratio of 0.8 to 0.2 approximately (Burke et al., 2002; Holmes et al., 2002; Ulyatt, 1997). The low cost associated with the management of these pasture-based systems represents a competitive advantage for the NZ agricultural export industry, with about 90% of milk production being exported (Holmes et al., 2002; Valentine & Kemp, 2007). However, there are several limitations imposed by pasture feeding; in particular the relatively low energy content in these forages compared with their high concentration of crude protein (CP), which is a major concern if it is not utilised efficiently (Ulyatt, 1997). The introduction into these systems of perennial ryegrasses with a higher content of water soluble carbohydrates (WSC), called 'High Sugar Grasses' (HSG), provides a potential solution to this problem.

Plant protein is rapidly degraded in the rumen, yielding amino acids (AA) and ammonia that the microbial population can utilise for growth. However, in temperate fresh forage diets, the rate of ammonia release usually exceeds the rate of microbial utilisation. Excess ammonia is absorbed from the rumen, detoxified in the liver, converted into urea and excreted in urine by the kidneys (Leng & Nolan, 1984). This represents a source of environmental pollution and a loss of protein that could otherwise be used by the animal for productive purposes.

If sufficient energy, which is mainly derived from soluble carbohydrate (CHO) fermentation, is provided, then AA and ammonia can be used by the microbes and converted into microbial protein, which will be then absorbed in the small intestine and is readily available for animal production (Kingston-Smith & Theodorou, 2000). When energy from soluble carbohydrate is not available in the proportion that is required, AA are used as energy yielding sources by microorganisms and this leads to great amounts of ammonia being accumulated in the rumen (Miller et al., 2001).

The aim of this Literature Review is to summarise feeding systems for dairy cows in NZ and to discuss nitrogen (N) and CHO digestion in the rumen. Particular attention will be given to the results obtained through several years of research on the utilisation of perennial ryegrasses with higher content of WSC on dairy pasture-based systems.

1.2 Dairy production and grazing systems in New Zealand

1.2.1 Dairy production in New Zealand: Main features

Pastoral dairy systems in NZ are based mainly on the utilisation of perennial ryegrass and white clover which are combined in a proportion of 0.8: 0.2, respectively. Clover is included in the diet due to its high feeding value (FV) and natural N fixation capacity. Although it can provide most of the N required by the pasture, the use of N fertiliser has increased in recent years. The average N fertiliser application on a NZ dairy farm is 110 kg per ha per year (Valentine & Kemp, 2007).

The rationale behind these pastoral systems is to synchronise the time of maximum feed requirements (early lactation) with the maximum rate of pasture production on the farm (spring) (Figure 1.1). The majority of dairy farms in NZ have established a pattern of seasonal calving to achieve synchrony between feed demand and feed supply. In this way, cows are calved in early spring (July and August, in northern and southern regions, respectively), lactate until late summer or autumn, depending on feed availability, and are dried-off before winter, in April or May. To achieve these targets, cows may conceive in October or November (Holmes et al., 2002). A small proportion of dairy farms have split-calving, which means that a reduced percentage of cows in the herd are mated to calve in autumn and supply milk to the industry during winter (Holmes et al., 2002).

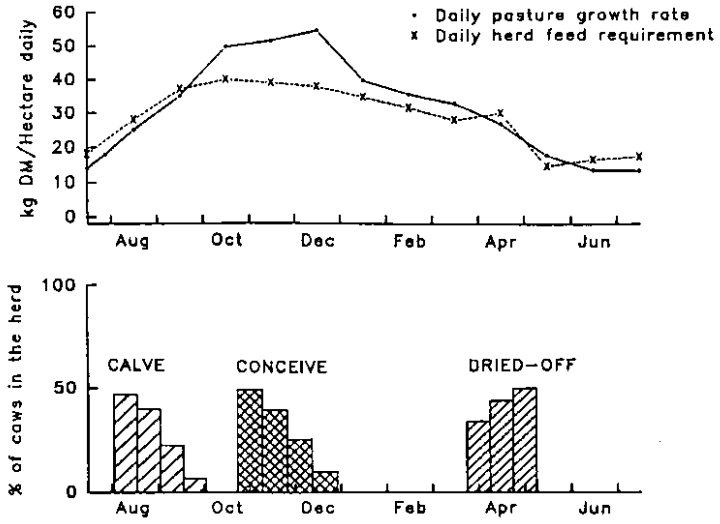

Figure 1.1: Seasonal calving pattern in NZ dairy farms (Holmes & Roche, 2007).

The use of supplements is only considered when the price of milk allows doing so. The ratio milk value: cost of supplements is usually the factor that determines the amount of supplements fed to the herd (Holmes et al., 2002; Holmes & Roche, 2007). The primary use of supplements is to fill a period of feed shortage, such as during dry summer conditions, although it can also be used to improve animal performance (Clark & Woodward, 2007; De Ruiter et al., 2007). Common supplements used in NZ include conserved forages such as hay, pasture silage, maize (*Zea mays)* silage, perennial and annual forage crops (*Brassica sp*., Lucerne; *Medicago sativa,* chicory; *Cichorium intybus*), and by-products such as palm kernel extract (PKE) (De Ruiter et al., 2007).

Silage is the preferred method for conserving pasture in NZ as it provides good quality feed for times of shortage at a relatively low cost (Holmes & Roche, 2007). Pasture hay is usually fed to dry cows in winter to substitute pasture and save it until spring (Holmes et al., 2002). Maize silage is used mainly in summer. It provides moderate levels of metabolisable energy (ME); however it is low in protein. *Brassica sp.* such as kale, turnips or rape, provide high quality feed with digestible leaves and bulbs and they can be used to fill summer and winter feed deficits**.** Palm kernel extract is a by-product of the palm oil industry and is imported in big quantities into NZ,

mainly from Asia. It can provide good levels of ME and reasonable levels of protein, but it is not highly palatable (De Ruiter et al., 2007).

According to the NZ national dairy statistics (LIC, 2008/2009), the NZ dairy industry comprises 11,618 dairy herds with 4,252,881 dairy cows. Total herd numbers have declined at a rate of 176 herds per year since 1978/79. However, the average herd size has increased for the last 30 seasons and is now 366 cows. An average dairy herd in NZ is farmed on 131 effective hectares, with a stocking rate of 2.83 cows/ha. Milk production averages 4,043 litres per cow in a relatively short lactation period of 266 days. Milk solids (MS) production per cow has increased in recent years, mainly due to genetic improvement and improved farm management and is now 323 kg MS/cow/year. Cows' breed is diverse, being 45% Holstein Friesian, 33% crossbreed (Holstein Friesian x Jersey), 14% of Jersey and a small proportion of other breeds such as Ayrshire.

During the season 2008/09, dairy companies processed 16 billion litres of milk containing 1.39 billion kg of MS. Total MS processed increased 9.7% compared with the previous season. The average dairy company payout for the season was NZ $5.57/kg MS.

1.2.2 Dairy grazing systems: advantages and limitations

The pastoral grazing system represents the main strength in the NZ dairy industry as it provides the cheapest feed to dairy cows (Holmes et al., 2002; Ulyatt, 1997; Valentine & Kemp, 2007), with pasture providing almost 90% of the feed required by the animal while the rest is usually supplied by pasture silage and/or hay (Holmes et al., 2002) or other supplements available. The other advantage of pastoral systems is that cows remain on pasture throughout the year, promoting huge savings in buildings, machinery and labor, when compared with many north hemisphere dairy systems. However, the necessity of the synchrony between feed demand and pasture growth reduces the length of lactation unless supplements can be incorporated into the system

(Pinares & Holmes, 1996). Furthermore, this heavy dependence on pasture growth makes the whole industry highly dependent on weather conditions.

The seasonal calving pattern that characterises most dairy farms in NZ allows farm staff to concentrate on one activity at a time, for example heat detection and mating, for seven weeks per year. The dry period also allows staff to take holidays, set up machinery, fences, and facilities for the following milking season.

Good pasture management and rotational grazing systems benefit the dairy industry and represent the vital key to ensure good quality feed (Lambert et al., 2004). In a rotational grazing system, pasture is offered to animals for a short period of time, animals are then removed for longer periods to allow pasture to regrow and be available for grazing again. Intensive grazing benefits pasture as it prevents lower parts of the plant from being shaded by taller parts and tiller density may be increased (Hodgson, 1990). Pre and post grazing herbage mass is the most important aspect of sward management.

When pre grazing herbage mass is too low, animal intake is restricted and animal performance, reduced. However, if it is too high, some parts of the plant will be aging and the quality of pasture will be poor (Holmes et al., 1992; Holmes et al., 2002; Holmes & Roche, 2007). A pre grazing herbage mass of 2,500 kg dry matter (DM) per ha is recommended to achieve high pasture intakes and maximise milk yield (Holmes & Roche, 2007). Post grazing residuals determine the quality of pasture for future grazings. Residuals of 1,500 kg DM/ha are recommended to maintain good quality pastures (Holmes & Roche, 2007).

The NZ dairy industry relies on its capacity to grow good quality and high yielding pastures throughout the year. This dependence on pasture growth has led to the development of good pasture management which allows farmers to sustain a high stocking rate and produce adequate levels of MS to achieve the industry targets (Lambert et al., 2004; Valentine & Kemp, 2007).

1.2.3 Feeding value of temperate forages used in New Zealand

Feeding value is defined as the animal production response to grazing a certain forage or diet, under unrestricted conditions, meaning that voluntary feed intake (VFI) is not limited by herbage availability (Ulyatt, 1981). The FV of a forage is a function of the forage nutritive value (NV) and VFI, measured in terms of milk production for dairy cows or liveweight (LW) gain for growing animals. The nutritive value of a forage is a function of its digestive characteristics and the efficiency of utilisation of absorbed nutrients (Ulyatt, 1981). It can be estimated by chemical analysis and indoor animal feeding trials (Waghorn & Clark, 2004). The FV of forages may vary widely considering different factors that affect their chemical composition and can be expressed as:

Feeding Value = NV x VFI

NV = proportion of nutrients digested x efficiency of utilisation of absorbed nutrients

As a rule, legumes have usually better FV than grasses and temperate forages have higher FV than tropical ones (Minson, 1981). Differences in terms of FV are also observed within species. Waghorn & Clark (2004) summarised a series of experiments where MS response in dairy cows fed perennial ryegrass was compared with other forages, as sole diets or combined with supplements.

Table 1.1: Milk solids (MS) response from cows in mid–late lactation fed different diets relative to performance of cows fed on perennial ryegrass. Adapted from Waghorn & Clark (2004).

Ryegrass versus:	Supplements (% dry matter intake)	MS response (%)
Cocksfoot[1]	-	-22
White clover	-	+9
White clover	25	+22
White clover	50	+29
Lotus[2]	-	+68
Chicory	40	+9
Turnips	30	+23
Pasture silage	25	0
Maize silage	25	0

[1] *Dactilis glomerata*
[2] *Lotus corniculatus*

Temperate species, grasses and legumes, have usually high digestibility throughout the year, although there are seasonal variations related to herbage maturity. As the plant matures over summer, the proportion of stems increases markedly and at the same time, their digestibility decreases, while digestibility of leaves is usually constant over the year (Litherland & Lambert, 2007). This double effect of reduced stem digestibility and increased stem proportion decreases the NV of temperate herbage in summer (Ulyatt, 1981; Waghorn & Barry, 1987). An important difference between grasses and legumes is that maturation has relatively minor effects on the composition and NV of legumes while changes in grasses are greater (Waghorn & Clark, 2004), as legumes usually maintain a higher leaf:stem ratio compared with grasses at the same stage of maturity (Waghorn & Barry, 1987).

Changes in plant cellular composition also affect the NV of forages. The proportion of cell walls and cell contents and cell wall digestibility can largely explain changes in sward quality. Legumes usually have less cell walls (so less fibre content) and higher cell content than grasses. As a result, digestibility is also higher (Waghorn et al., 2007). Another factor that explains the higher FV of legumes over grasses is the efficiency of utilisation of absorbed nutrients. Ruminants utilise

dietary nutrients with different efficiencies. Efficiency of utilisation of ME above maintenance is greater for white clover than for perennial ryegrass (Rattray & Joyce, 1974).

Herbage intake can also explain the greater FV of legumes. Legumes have a faster rate of passage, meaning that they are retained for less time in the rumen than grasses. This is due to the higher ratio of readily fermentable: structural carbohydrate in legumes. Voluntary feed intake is thus higher for legumes, as the rate of clearance of digesta from the rumen is faster than for grasses (Ulyatt, 1981).

New Zealand farmers now have access to a wide variety of forages. Ryegrasses are now available with different endophyte status, diploid or tetraploid, early or late flowering (Moot et al., 2007), and also as hybrid and Italian ryegrasses. Novel endophyte strains have been selected to reduce the negative impact of endophytes on pasture quality such as ryegrass staggers and heat stress in livestock produced by the toxins lolitrem B and ergovaline, respectively. A common example is the endophyte AR1 or AR37 selected from a collection of European ryegrass endophytes with the purpose of avoiding animal health problems while maintaining positive effects on plant persistence in NZ ryegrass cultivars (Tapper & Latch, 1999). Ryegrasses infected with novel endophytes produce only traces amounts of lolitrem B and ergovaline but substantial quantities of peramine (AR1) or epoxyjanthitrem (AR37), thus retaining a defense against attack by insects.

Plant breeding and selection have also made available cultivars which differ in the date of flowering, determining sward quality. As grasses mature and they are close to flowering, there is an increase of stem compared with leaf and this diminishes their NV. Annual tetraploid ryegrasses (*Lolium multiflorum*) are more digestible than perennial ryegrass as they contain more cell content and less cell wall; in the same way, perennial tetraploid ryegrasses are more digestible than diploid ryegrasses, increasing animal pasture intake (Litherland & Lambert, 2007; Valentine & Kemp, 2007). Italian and hybrid ryegrasses are also known to have a better FV

compared with perennial ryegrass. They can provide highly digestible feed and high yields of DM in winter and early spring (Valentine & Kemp, 2007). As a general rule, ryegrasses that have increased FV for the reasons discussed before also tend to have reduced persistency compared with normal diploid perennial ryegrasses infected with unselected wild endophyte.

1.3 Nitrogen and carbohydrate metabolism in the rumen

Protein and CHO are the major nutrients required in the diet of ruminants. The requirement for these specific nutrients in dairy cows is determined by milk yield and composition, maintenance requirements, stage of pregnancy, and mobilisation of body tissues (LW gain or loss)(Kolver, 2003). To optimise milk production these requirements must be matched with dietary supply. Ruminants have the ability to utilise fibrous feeds (to supply their energy and protein requirements) due to the presence of microorganisms in the rumen which are able to digest such feedstuffs (Lapierre et al., 2004; McDonald et al., 2002; Van Soest, 1994). Dietary ingredients that pass through the reticulo-rumen are partially digested and used for microbial growth. In this way nutrients available for absorption differ from those present in the diet, making it difficult to predict nutrient supply (Lapierre et al., 2004). Amino acid requirements in ruminants are met from microbes grown in the rumen and digested in the small intestine and from dietary protein that is not degraded in the rumen but can be digested in the small intestine ('by-pass' protein)(Leng & Nolan, 1984). On the other hand, the supply of energy for the microorganisms in the rumen comes from the fermentation of soluble CHO present in the feed. The main products of the CHO fermentation process are volatile fatty acids (VFA; acetic, propionic and butyric) as well as methane and carbon dioxide. Volatile fatty acids provide almost two thirds of the total amount of ME that ruminants gain from their diets (CSIRO, 2007). Microbial cells are also an important source of ME for the ruminant (CSIRO, 2007).

1.3.1 Major transactions of carbohydrates in the rumen

Carbohydrates are the main component of plant tissues and the major source of energy for the ruminant. Water soluble carbohydrate and pectins constitute what is called the readily fermentable carbohydrate fraction, which is completely fermented by microbes in the rumen and converted into VFA (McDonald et al., 2002). Seventy to 90 % of cellulose and 60 to 90% of hemicelluloses present in plants can also be digested by cattle (Waghorn & Barry, 1987). Sugars

from the digestion of CHO are metabolised intra-cellularly by the microbes and produce pyruvate, which is further converted to VFA releasing carbon dioxide, methane, and also energy in the form of adenosine triphosphate (ATP)(McDonald et al., 2002; Van Soest, 1994). Further microbial digestion of CHO can occur in the large intestine (Waghorn & Barry, 1987). The relative proportions of individual VFA released in the rumen depend on the diet. In pasture or forage diets the molar proportion of VFA produced ranges from 60 to 72% for acetic acid, 15 to 23% for propionic and 12 to 18% for butyric acid. When large amounts of grain are fed, a higher proportion of propionic acid is formed relative to the other two VFA (Holmes et al., 2002) (Figure 1.2).

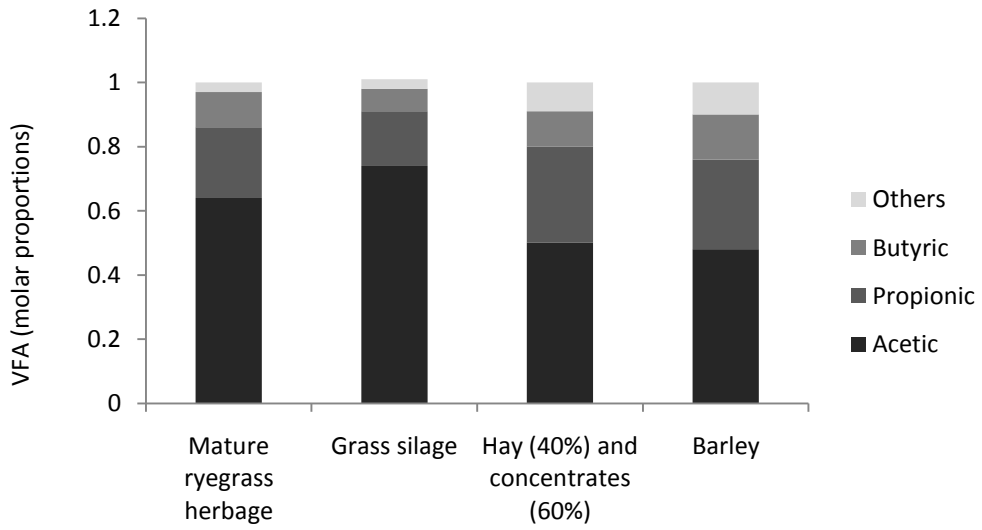

Figure 1.2: Volatile fatty acids production in cattle fed different diets. Data from McDonald et al., (2002).

A lactating dairy cow can produce 3 to 5 kg of VFA per day (Holmes et al., 2002). The concentration of VFA in the rumen fluids may vary between 50 to 150 mM/litre depending on the diet fed. The majority of VFA are absorbed from the rumen by direct diffusion (although 10% of VFA can be produced and absorbed in the large intestine) and transported to the liver by the portal bloodstream (Holmes et al., 2002). After digestion and absorption, nutrients undergo a series of chemical processes and may supply the precursors for the synthesis of milk or body tissue or may be degraded to provide energy to meet animal maintenance requirements.

1.3.2 Major transactions of nitrogen in the rumen

The main sources of N in the diet of a ruminant are AA and peptides which result from the rumen fermentation of ingested plant proteins. Ammonia is the immediate end-product that results from protein catabolism and it is toxic to the animal cells if it is allowed to accumulate in the organism (Stewart & Smith, 2005). Major N transactions in the rumen are shown in Figure 1.3. Nitrogen inputs are represented by dietary and recycled N. Dietary protein can be further divided into rumen degradable (RDP) and undegradable protein (UDP). Rumen degradable protein comprises non protein nitrogen (NPN) and dietary true protein. True protein is degraded to peptides and AA, which can be deaminated into ammonia or incorporated into microbial protein. Non protein N is used for microbial growth and it is composed of N present in ammonia, urea, AA, and small peptides. Nitrogen outputs are represented by ammonia, undegraded protein (dietary or endogenous) and microbial protein. When the amount of dietary RDP exceeds that required by the ruminal microorganisms, the protein is degraded into ammonia, transformed into urea in the liver and excreted as urine (Bach et al., 2005). This is the typical situation that occurs in NZ in animals fed fresh temperate pasture. A principal factor limiting microbial growth in the rumen is the low concentrations of readily fermentable CHO (10 - 15% DM) (Burke et al., 2002), relative to CP, which provides energy for microbial growth in the rumen.

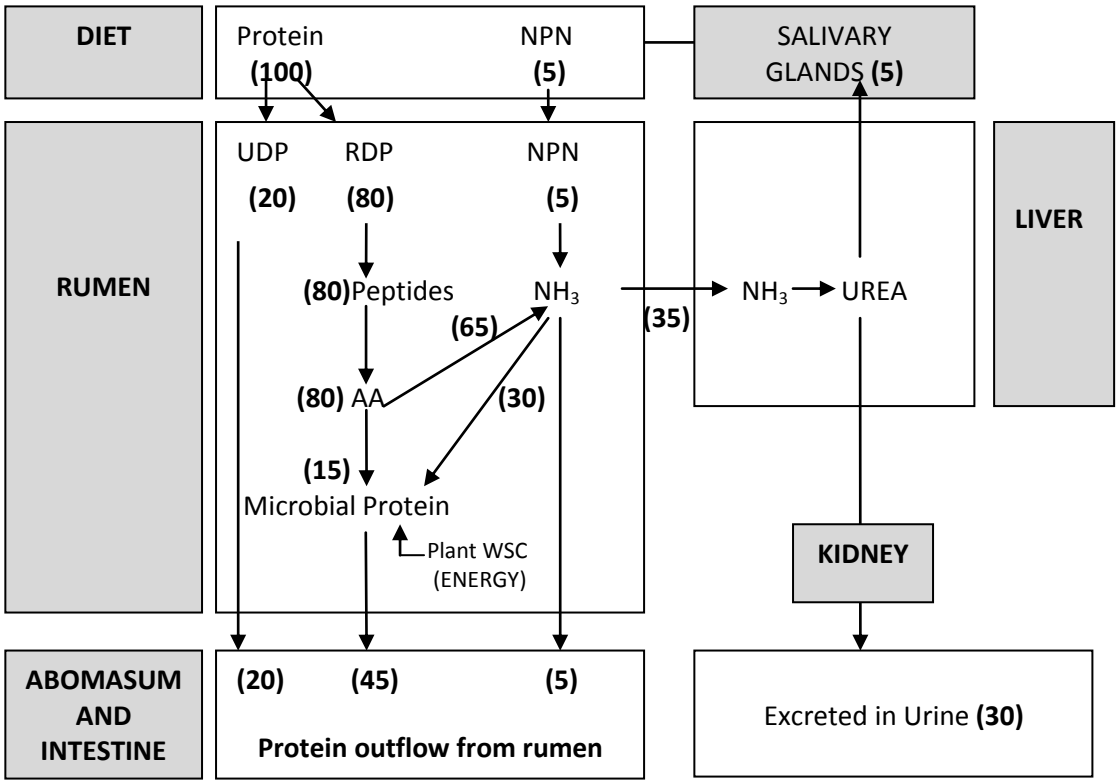

Figure 1.3: Schematic representation of nitrogen (N) flows in the rumen relative to 100% of the total N eaten, using New Zealand data from feeding fresh forage diets. Adapted from Leng & Nolan (1984); MacRae & Ulyatt (1974); McDonald et al., (2002). Microbial protein synthesis is critically dependent upon the supply of fermentable energy from water soluble carbohydrates (WSC), as well as the supply of ammonia (NH_3) and amino acids (AA).

From Figure 1.3 it can be concluded that 80% of pasture N is degraded by microbes in the rumen, while the rest is considered to be undegradable and passes through the rumen as 'by-pass' protein. From that 80% of RDP, approximately 65% is converted to ammonia, which can be further incorporated into microbial protein (30%) or can be absorbed through the rumen wall (35%) and be converted into urea in the liver. From that 35%, a significant proportion (30%) is excreted in urine and the rest (5%) is recycled via saliva.

1.3.3 Protein breakdown in the rumen

Nitrogen metabolism in the rumen can be divided into two different processes: protein degradation, which provides N sources for bacteria, and protein synthesis, which provides the AA required for animal products (Bach et al., 2005). Dietary protein breakdown is a complex process

that involves different microorganisms (including bacteria, protozoa and fungi) which provide the enzymes that are necessary to hydrolyse peptide bonds. The hydrolysis of proteins results in oligopeptides and is performed by bacterial proteases and peptidases. Oligopeptides are then broken down into smaller peptides and finally converted to AA, which are incorporated into microbial protein or further deaminated to ammonia (Schwab et al., 2005; Wallace, 1994). Proteases contained in plant cells perform the initial phase of proteolysis in the first few hours after the ingestion of fresh forages (Theodorou et al., 1996). Proteases and peptidases are present in significant quantities in mature leaves and are further induced during stress and senescence. Following disruption of plant cells by chewing, leaf cells possess the intrinsic capacity to degrade their own proteins prior to invasion by microorganism enzymes (Kingston-Smith & Theodorou, 2000).

Protein breakdown provides these microbes with AA and peptides necessary for their growth. However, in conditions where dietary protein is abundant, an excessive production of ammonia can occur, contributing to an inefficient N retention and utilisation (Leng & Nolan, 1984; Oba et al., 2005). Several factors affect microbial protein degradation including: type of protein, ruminal dilution rate and ruminal pH (Bach et al., 2005).

Solubility of proteins is a key factor that determines their degradability. As an example, prolamins and glutenins are insoluble and thus slowly degraded, but globulins are highly soluble and degradable (Romagnolo et al., 1994). The structure (tertiary and quaternary) of the protein is also important when considering its solubility. In addition, some specific peptide bonds are more resistant than others to degradation. Another factor that determines protein degradability is the ruminal dilution; an increase in passage rate would result in a reduction in protein degradation causing an increase in the flow of undegraded dietary protein supply to the small intestine. Additionally, protein degradation is also affected by ruminal pH and the type of ration fed to

animals, as this establishes the type of bacteria that will predominate in the rumen (Bach et al., 2005).

1.3.4 Ammonia production in the rumen

Ammonia is an important source of N for the growth of the microbial population in the rumen (Abdoun et al., 2006). Ammonia is produced in the rumen as a result of two main processes: microbial degradation of proteins, peptides, AA and nucleic acids; and also as a result of microbial hydrolysis of urea passing across epithelia of the gastrointestinal tract from the blood and interstitial fluids, and urea recycled into the rumen via saliva (Abdoun et al., 2006). Ammonia N is lost from the rumen by the incorporation into microbial cells that pass out of the rumen, by absorption through the rumen wall (35-65% of the total ammonia can be lost in this way), and it is also irreversibly lost in the fluid passing out of the rumen but the proportion is relatively small, only 10%) (Leng & Nolan, 1984).

Several experiments have demonstrated the impact of forage-based diets on rumen ammonia concentration within various time periods after feeding. In the case of silage feeding, these effects are pronounced because the soluble nitrogenous components (present in silage) are rapidly degraded in the rumen and this results in peaks of ammonia concentration of 18-20 mM within 1 hour after feeding, from basal levels of 2-4 mM. This level can be attenuated by chemical treatment to reduce N solubility or by providing a readily fermentable CHO source to provide energy for N capture by the rumen microflora (see section 1.3.5). In the absence of sufficient energy supply, rapid fluctuations in ammonia concentration may occur.

An experiment conducted by Dewhurst et al. (1999) showed that dairy cows offered grass silage diets had poor conversion efficiency of feed N to milk N, with values ranging from 23 to 32%. When legume silages (with higher N content) were used, even lower efficiencies were observed (18% for lucerne silage) (Dewhurst et al., 1999). This suggests that, despite the great importance

of ammonia in the growth of rumen microorganism, dairy cows cannot utilise all the ammonia produced because of a deficiency in energy from readily fermentable CHO.

When rumen ammonia is produced in excess to the ability of the microbes to assimilate it, it is absorbed in the blood, carried into the liver and finally converted into urea. Most of the urea formed in the liver is excreted through the kidney into urine and a smaller part is recycled back into the rumen via saliva or through the rumen wall (Leng & Nolan, 1984). In the rumen it is decomposed by bacterial urease to ammonia which is utilised in microbial protein biosynthesis with the ammonia produced from degradation of dietary protein. This decomposition of urea is carried out, in a small proportion, by ureolytic bacteria present in the rumen, but in a greater extent by the bacterial population adhering to the surface of the rumen wall (Stewart & Smith, 2005). These bacteria intercept the urea that passes across the rumen wall from the blood. The entry of urea into the rumen is allowed when ruminal ammonia concentration is low. Only under these conditions, high levels of endogenous N recycled are able to serve as a secondary source of N for the ruminal microorganisms and therefore represents a N saving mechanism (Bondi, 1987; Stewart & Smith, 2005).

Rumen microbial protein synthesis achieves a maximum level when the concentration of ammonia in the rumen contents reaches 5-6 mM (Pisulewski et al., 1981). Further increments of N intake gradually increase rumen ammonia concentration but total microbial protein production attains a maximum rate. Ammonia levels above 5-6 mM will not further increase microbial protein production, causing inefficient use of N in the rumen with the consequent loss of ammonia from the rumen by absorption across the gut wall, thereby reducing both energy and protein supply to the host animal. This concentration of ammonia in the rumen is attained with diets containing 13 % of CP in the DM (Pisulewski et al., 1981). In NZ pastoral systems, the CP content of vegetative pasture (25 to 30% DM; Burke et al., 2002) is higher than the value

proposed, so protein exceeding these requirements is wasteful and leads to excess rumen ammonia concentration and thus, an inefficient N retention.

1.3.5 Microbial protein synthesis

Over half of the AA absorbed in the small intestine of ruminants comes from microbial protein (AFRC, 1993). Hence, it is essential to quantify the amount of microbial protein synthesised in the rumen to improve the efficiency of N utilisation and make a better use of the N contained in forages. Daily microbial protein yield is the product of microbial efficiency, defined as microbial N synthesised per kg of organic matter (OM) fermented in rumen, and the total kg of OM fermented in rumen per day (Hoover & Stokes, 1991).

Adequate supplies of fermentable CHO and N sources (AA and ammonia) are needed for microbial growth. The yield of microbial biomass depends on the amount of substrate available and the energy used for maintenance by the microbes. Because of the complexity of the rumen and also due to technical difficulties, microbial protein synthesis (MPS) is not easy to estimate *in vivo*. Dietary factors such as level of feeding, rumen synchrony, and forage quality are considered to affect MPS (Dewhurst et al., 1999). It is well known that MPS is maximised by synchronising the availability of fermentable CHO and degradable N in the rumen. This synchrony between energy derived from the VFA and absorption of metabolisable protein (MP) is essential to maximise the conversion of dietary protein into milk protein or other animal products. Figure 1.4 shows the typical situation for ruminants fed fresh pasture, where protein in fresh forage diets is degraded faster than CHO. This means that in an environment where energy is limited but there is an excess of peptides and AA from plant cell degradation, microbes in the rumen will use AA as a source of energy and as a result of this deamination process, great amounts of ammonia will be released into the rumen (Kingston-Smith & Theodorou, 2000). Ammonia production in excess, i.e. that cannot be assimilated by microbes, can account for 35 % of the N eaten (Beever et al., 1986),

most of which is eliminated in urine. Increasing the WSC of the diet could potentially balance protein and energy supplies.

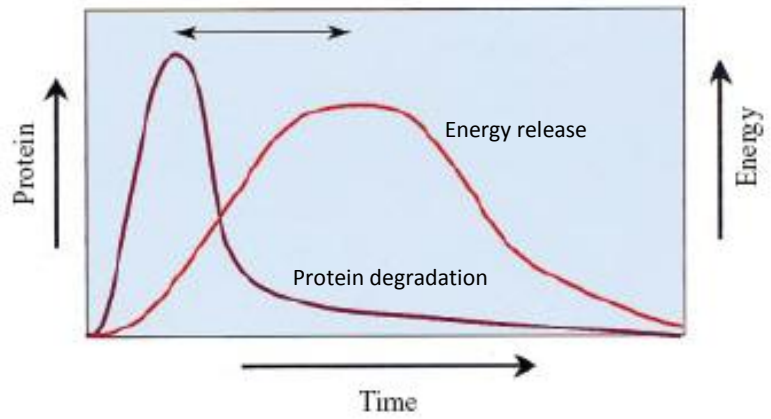

Figure 1.4: Asynchrony in forage degradation by ruminants. A schematic representation of the mismatch in the timing of peaks of protein degradation and energy release (Kingston-Smith & Theodorou, 2000).

An experiment was conducted in NZ to test the 'asynchrony hypothesis' presented in Figure 1.4 (Kolver et al., 1998). A supplement with high concentration of WSC was fed to dairy cows at the same time that pasture was fed (synchronous diet), or 4 hours after pasture (asynchronous diet). The study measured the changes in ammonia concentration in the rumen as an indicator of MPS. Cows fed the synchronous diet showed that peak of ammonia concentration, at 3 and 5 hours after pasture feeding, was reduced by approximately 33% of values obtained from cows fed the asynchronous diet. Based on the results, it was concluded that the synchronous release of supplemental CHO with pasture N seems to improve the capture of ruminal N by microbes. However, these changes did not affect the N status or milk production of the cows.

1.3.6 Ammonia detoxification in the rumen

The N that cannot be 'captured' by microbes is converted to urea in the liver to detoxify the excess ammonia. There is a metabolic cost associated with conversion of ammonia to urea, which requires energy (Muller, 2002; Ulyatt, 1997). The energy used in this process will not be available for milk production or body tissue and as a result, a reduced animal performance is expected.

It was suggested that there is an energy cost of up to 4% of ME intake associated with the synthesis of urea in the liver, but a potentially greater cost in detoxifying excess ammonia (Waghorn & Wolff, 1984). In this way, a NZ Holstein Friesian cow producing 1.5 kg MS/day will require approximately 15.9 kg DM/day to meet its requirements (Holmes et al., 2002). Considering that a typical ryegrass and white clover fresh forage diet in NZ contains 11 MJ ME/kg DM (Burke et al., 2002), the 'urea cost' of that particular cow would be 7 MJ ME/day. To produce a litre of milk (4.5 % fat and 3.5% protein) would cost 5 MJ ME (Holmes et al., 2002), thus the 'urea cost' would be 1.4 litres of milk per cow per day.

Lobley et al. (1995) found, in studies using sheep fed lucerne pellets, that hepatic removal of ammonia appears to require the net utilisation of considerable amounts of absorbed protein. This would result in a lower proportion of absorbed AA being available for muscle and milk protein synthesis. Blood urea concentrations in ruminants fed highly digestible fresh herbage are considerably higher than those produced by similar intakes of dried feed, which also suggest that the metabolic cost of detoxifying ammonia on diets of fresh herbage could be very high (Lobley et al., 1995).

Synchronisation of N and energy release within the rumen in order to maximise nutrient capture by microbial populations has been an objective in ruminant feeding systems (Abdoun et al., 2006). This alternative to improve N utilisation by the animal is discussed in section 1.5, where the effects on ruminant production, of incorporating grasses with a higher content of WSC are reviewed.

1.4 Nitrogen partitioning within the cow

1.4.1 Milk production and nitrogen utilisation

Amino acids in dietary protein escaping degradation in the rumen (UDP) and those contained in the microbial protein fraction, are absorbed from the small intestine and together deliver MP to the organs and tissues of the dairy cow (CSIRO, 2007; Holmes et al., 2002; Tamminga, 2005). The AA profile of MP supplied to the tissues does not always match that of the protein to be formed by the cow, so the synthesis of milk (or other animal product) will be limited by the quantity of the most limiting essential AA. Other AA in excess will be catabolised and converted to urinary urea (Holmes et al., 2002; Tamminga, 2005).

Protein N secreted in milk depends on the yield and protein concentration, which varies markedly between breeds. Typical NZ values are 4.2%, 3.5% and 3.7% of protein in milk for Jersey, Holstein Friesian and cross-breed cows (Jersey x Holstein Friesian), respectively (Holmes et al., 2002). If dairy cows are losing weight (as occurs at the beginning of the lactation), mobilised tissue contributes AA that can be used in the mammary gland for milk synthesis. As an example, an adult cow can provide 138 g MP per kg of mobilised tissue (AFRC, 1993), which may be sufficient to synthesise 2.5 to 3 litres of milk (4.5% fat and 3.5% protein) (Holmes et al., 2002).

In fresh forage diets, ME intake is usually the factor that limits milk production (Burke et al., 2002; Waghorn & Clark, 2004), and together with the supply of protein to the duodenum have been reported to limit milk production to 25 litres per day in animals consuming fresh forage diets (Beever & Siddons, 1986). An experiment conducted by Kolver & Muller (1998) predicted the utilisation of N in grazing cows fed a good quality fresh forage diet and a total mixed ration (TMR). Results are shown in Table 1.2.

Table 1.2: Estimated nitrogen (N) utilisation of cows grazing a fresh forage diet and cows fed a total mixed ration (TMR) (Kolver & Muller, 1998).

	Pasture	TMR
Dry matter intake (kg/day)	19.0	23.4
N intake (kg/day)	0.79	0.75
Milk production (kg/day)	29.6	44.1
Gross N efficiency (g milk N/100 g dietary N)	16.2	27.3
Predicted 'urea cost'(MJ ME/day)[1]	12.45	4.18
Predicted efficiency of microbial protein synthesis[1] (g N/kg organic matter truly digested in the rumen)	27	31
Predicted microbial flow (g N/day)[1]	328	371

[1]Values predicted using the Cornell Net Carbohydrate and Protein System model.

Cows fed on the fresh forage diet produced 14.5 kg of milk less than those cows in the TMR ration. Although daily N intake was similar for both treatments, cows eating pasture had a lower dry matter intake (DMI) compared to TMR cows, which suggests that the fresh forage diet had higher content of CP (26% DM versus 20% DM). Compared to the TMR cows, grazing cows were less efficient in converting dietary N into milk N, and as a result excreted more N in urine as suggested by the higher metabolic 'urea cost'. The model also predicted lower efficiency of MPS and microbial protein flow for the grazing cows, which together explained the lower milk production. Non structural CHO represented 19.3% DM and 28.8% DM for fresh pasture and TMR, respectively. The lower percentage of dietary soluble CHO may partially explain the lower milk production for grazing cows, which suggest the importance of balancing readily fermentable carbohydrates (WSC) and CP in dairy cows' diet.

1.4.2 Nitrogen excretion and its impact on the environment

Farming in NZ has been identified as an important source of N pollution for the environment, with dairy cattle being major contributors (De Klein & Ledgard, 2001). Contamination of ground

and surface waters by nitrate leaching is a serious consequence of dairy farming. The main cause of nitrate leaching in dairy farms with grazing cows is the deposition of N in small concentrated urine patches (De Klein & Ledgard, 2001; Johnson & Baldwin, 2008), which are also the main source of nitrous oxide emissions, a harmful greenhouse gas (Luo et al., 2008).

The quantities of N that are deposited in urine patches exceed the immediate plant requirements, leaving the excess N subject to leaching losses and nitrous oxide emissions (Haynes & Williams, 1993; Pacheco & Waghorn, 2008). Dung patches may also contribute to N pollution, but to a lesser degree, as dung consists mainly of organically bound N that is released slowly (De Klein & Ledgard, 2001; Haynes & Williams, 1993). Increases in N of 20–80 g N/ m^2 in urine patches can be expected from animal urine. Typically, over 70% of the N in urine is present as urea that undergoes processes in the soil to produce ammonium ions, which are subjected to nitrification and subsequent denitrification (Haynes & Williams, 1993).

Enhancing rumen N efficiency may result in a more efficient conversion of plant protein into milk N with considerable benefits to the environment (Kingston-Smith & Theodorou, 2000), contributing to the sustainability of the dairy industry (Edwards et al., 2007; Johnson & Baldwin, 2008).The use of pastures with elevated concentration of WSC needs to be evaluated for achieving this goal.

1.5 Development of High Sugar Grasses as an alternative to improve nitrogen utilisation in the rumen

Using cultivars with increased content of WSC may benefit the dairy industry in two ways. Firstly, it has been hypothesised that HSG may increase milk production due to a higher digestible DMI per cow. Secondly, it may also improve the efficiency of N capture in the rumen, increasing the supply of N to the small intestine (and AA) and so improving milk production and reducing the

amount of N that is excreted to the environment through a better utilisation of dietary N (Kingston-Smith & Theodorou, 2000; Miller et al., 2001).

Potential benefits for the dairy industry can be observed in Figure 1.5. As the dairy industry size grows, by increasing the stocking rate or the amount of N fertiliser applied, there is also an increase in the 'environmental impact' associated with N emissions. If the hypothesised benefits of the HSG can be confirmed, there could be a reduction in the environmental impact for a given industry size. As an example, the dairy industry could grow from A to B without changing the levels of environmental impact. Or, environmental impact may be reduced from A to C and still maintain the same industry size (Edwards et al., 2007).

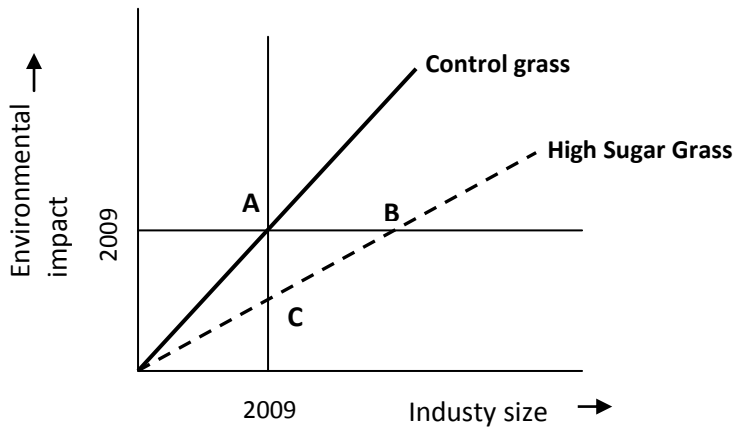

Figure 1.5: Opportunities to grow for the dairy industry while minimising nitrogen emissions. Adapted from Edwards et al., (2007).

In order to achieve these goals, a variety of ryegrasses bred to contain higher levels of WSC has been selected by traditional breeding methods. The first HSG cultivar was developed in Aberystwyth, Wales, by the Institute of Grassland and Environmental Research; IGER (now Institute of Biological, Environmental and Rural Sciences; IBERS) (Humphreys, 1989a, 1989b, 1989c) and it was named AberDove (initially, Ba11353) but it was never marketed (Edwards et al., 2007). The first HSG cultivar available in the market was AberDart (Edwards et al., 2007) and this was used for the experiments conducted in Chapter 2.

The development of this HSG focused on increasing the accumulation of high molecular weight fructans, although results from experiments reviewed here report total concentration of WSC (without discriminating between low and high molecular weight sugars). Several factors may affect the expression of WSC in the plant; these include the cutting regime, the amount of N fertiliser applied (Moorby et al., 2006), and the climate (Parsons et al., 2004), among the most important. Low temperatures are known to elevate the WSC in ryegrass, notably fructans, which may reach a peak of accumulation in mid winter, falling rapidly in spring, coincident with flowering (Edwards et al., 2007). An experiment was conducted in NZ to investigate possible genotype x environment interaction in the expression of the higher WSC trait in ryegrass (Parsons et al., 2004). The results showed that low temperatures at night and/or sustained low temperatures (less than 10^{o}C) previous to harvesting the grass are necessary for expressing a sustained higher WSC content in the grass. Nitrogen fertiliser has also been shown to decrease concentrations of WSC (Miller et al., 2000) and increase concentrations of CP in the plant.

1.5.1 Studies in the United Kingdom and the Netherlands

Various experiments were conducted in the UK to investigate the effects of feeding cows with HSG on aspects of N partitioning in dairy cows. An experimental ryegrass (AberDove, never marketed) was tested against a control ryegrass in early (Moorby et al., 2006), mid (Miller et al., 2000) and late (Miller et al., 2001) lactation dairy cows, however these studies were not carried out in the same lactation or year. Cows were stall fed with perennial ryegrass cut from the field and an additional 4 kg/day of concentrate was supplied during the three trials. In two of them management techniques were used to accentuate differences between the HSG and the control grass. Nitrogen fertiliser was applied to the plots of control grass to exaggerate differences in WSC concentrations between treatments (Miller et al., 2000), and the control diet was cut in the morning while HSG was cut in the afternoon for the same purpose in the trial conducted by

Moorby et al., (2006). All swards were of pure perennial ryegrass. Main findings are summarised in Table 1.3.

Table 1.3: Herbage chemical composition of high sugar grass (HSG; cv. AberDove) and control grass (cv. AberElan) and effects on milk production and partitioning of nitrogen (N) intake between milk and urine in three United Kingdom experiments.

	Early lactation (Moorby et al., 2006)			**Mid lactation (Miller et al., 2000)**			**Late lactation (Miller et al., 2001)**		
	HSG	Control	Sig[1]	HSG	Control	Sig[1]	HSG	Control	Sig[1]
Plant composition									
WSC (g/kg DM)	243	161	***	234	194	NA	165	126	*
CP (g/kg DM)	104	99	NS	107	145	NA	92	106	*
NDF (g/kg DM)	480	563	***	478	480	NS	544	589	*
Herbage DMI(kg DM/day)	15.3	12.5	**	14.6	14.8	NS	11.6	10.7	NS
Milk yield (kg/day)	32.7	30.4	NS	21.4	21.9	NS	15.3	12.6	*
N intake(g/day)	376	320	**	358	453	**	280	290	NS
N output urine (g/day)	75	87	NS	64	121	***	71.3	100	***
Proportion of N intake in urine	0.20	0.27	*	0.18	0.27	*	0.25	0.35	**
N output milk (g/day)	136	119	*	107	111	NS	83.3	67.5	**
Proportion of N intake in milk	0.36	0.37	NS	0.30	0.25	*	0.30	0.23	**

[1]Significance of treatment: NA; not available, NS; not significant, * P<0.05, **P<0.01, ***P<0.001

High sugar grass cultivars showed increased WSC content and reduced CP concentration in all three experiments. Additionally, neutral detergent fibre (NDF) concentration was reduced in two experiments and this was associated with an increased DMI by dairy cows in early lactation. There was no difference in DMI in the other two experiments. The use of HSG consistently decreased the proportion of dietary N which was excreted as urine in the three experiments and increased the proportion of dietary N secreted in milk in two of them. Milk yield was only increased by feeding HSG in one experiment, when it was fed in autumn to cows in late lactation, which produced 2.7 kg per day more milk than cows eating the control grass. Cows in early lactation fed the HSG diet also tended to produce more milk than cows in the control diet, although this difference was not statistically significant and was associated with the greater DMI of cows fed HSG (Moorby et al., 2006). Edwards et al., (2007) proposed that the positive

productive and environmental responses observed in some UK studies may be attributed to the low content of CP in the diets, suggesting that the animal was limited by protein intake rather than energy intake.

These experiments can be summarised as showing that feeding HSG improved the efficiency of converting dietary N to milk N, principally by reducing N partition to urine and increasing the partition of N to milk. It is evident that only in late lactation, cows offered the HSG diet increased milk yield significantly whilst the other two other experiments failed to confirm possible benefits in term of milk production. However, they showed evidence that feeding HSG reduced urinary N output from dairy cows and made pastoral dairy farming more sustainable for the environment.

Another study was conducted in the Netherlands (indoor feeding trial) to evaluate feeding HSG on milk output and urinary N excretion in mid lactation cows (Taweel et al., 2005). Six varieties of ryegrasses were tested and two of them were higher in WSC. It is important to highlight that HSG cultivars used in this study differed from UK HSG varieties. Differences in NDF and CP between varieties were only marginal. Results showed that milk yield and composition were not affected by the treatments. Concentration of WSC in the two HSG cultivars was 24 g/kg DM and 31 g/kg DM higher than the rest of the cultivars. These differences are lower than those reported by Miller et al., (2001). Dry matter intake was also similar between treatments. The authors concluded that the levels of differences between the HSG varieties and control varieties were not high enough to increase milk production or DMI.

Further studies were conducted using fistulated steers to test the effect of feeding HSG on rumen metabolism and N absorption from the small intestine (Lee et al., 2002; Table 1.4). Steers had *ad-libitum* access to one of the two varieties cut at different times of the day to accentuate differences in the concentration of WSC. Greater concentrations of WSC (82 g/kg) were found in the HSG variety compared with the control. *In vitro* dry matter digestibility (DMD) was also higher due to the lower NDF and acid detergent fibre (ADF) in the HSG cultivar. As a consequence, DMI

increased in those steers fed the HSG diet and contributed to a higher flow of NAN to the duodenum and increased AA absorbed from the small intestine. Rumen ammonia levels were lower and the ratio propionate: acetate higher for the HSG animals. However, there was no significant change in the efficiency of MPS on the HSG diet and the flow of microbial N to the duodenum per unit of N intake was similar in the two diets.

Pacheco et al., (2007) suggested that the ratio of CP:WSC in perennial ryegrass may be more important than the WSC concentration alone. This ratio may have to decrease below a certain range before major changes in animal performance can be expressed (see section 1.5.3).

Table 1.4: Herbage chemical composition and rumen parameters of fistulated steers offered a perennial ryegrass with elevated concentrations of water soluble carbohydrates (HSG; cv. AberDove) and a control perennial ryegrass (cv. AberElan) (Lee et al., 2002).

	Control	HSG	Sig[1]
Plant composition			
WSC (g/kg DM)	160.7	243.2	***
CP (g/kg DM)[2]	99.3	103.7	NS
ADF (g/kg DM)	295.7	251.4	***
NDF (g/kg DM)	562.5	479.6	***
***In vitro* dry matter digestibility**	0.56	0.61	**
DMI (kg/day)	6.7	9.3	***
Ammonia-nitrogen (mg N/litre)	26.4	14.0	***
Ratio propionate:acetate (mmol/litre)	0.26	0.30	***
Flow of duodenal non ammonia nitrogen (g/day)	98.5	128.9	*

[1]Significance of treatment: NS, not significant, * P<0.05, **P<0.01, ***P<0.001
[2]Calculated as Total N x 6.25

1.5.2 Studies in New Zealand

Experiments conducted in NZ in four consecutive years (spring 2004 and 2005 and autumn 2006 and 2007) investigated the impact of high WSC grass varieties on milk and MS yields (Cosgrove et al., 2007). These studies compared two ryegrasses with elevated concentrations of WSC (a diploid perennial ryegrass cv. AberDart; HSG, and a tetraploid annual Italian ryegrass cv. Moata; IRG) against a standard diploid perennial ryegrass (cv. Impact; STG) as the control. Each ryegrass was sown as a pure grass (clover-free). Spring trials were conducted over six consecutive weeks, while

the measurement period for autumn was only two weeks. Results for grass chemical composition, milk and MS yield are shown in Table 1.5.

In the two successive springs, HSG and IRG had similar concentrations of WSC and these were always higher, 20 to 40 g/kg DM higher, than the control (STG). Crude protein, in spring 2004, and structural fibre, in autumn 2006, were lower in the two high WSC cultivars compared with the control. Although higher concentrations of WSC were found in the two cultivars, no significant differences were detected in milk and MS yields in spring in both years. Milk yields tended to be greater for the HSG during autumn, but this was only significant in 2007. Mean WSC concentration was lower in autumn (160 g/kg DM) compared to spring (200 g/kg DM), whilst CP concentrations were higher during autumn (261 versus 225 g/kg DM). Cosgrove et al., (2007) suggested that with the low concentrations of WSC and high CP concentrations in autumn, "it is possible that cows may benefit more from smaller increments in WSC in autumn than they do in spring" (pp 184), suggesting the importance of the CP:WSC ratio.

Table 1.5: Herbage chemical composition and effect on milk production of STG: standard diploid perennial ryegrass; cv. Impact (control); HSG: diploid perennial ryegrass with higher concentrations of water soluble carbohydrates; cv. AberDart and IRG: tetraploid Italian annual ryegrass; cv. Moata fed to dairy cows in New Zealand (Cosgrove et al., 2007).

	Spring (2004)				**Spring (2005)**				**Autumn (2006)**				**Autumn (2007)**		
	STG	HSG	IRG	Sig[1]	STG	HSG	IRG	Sig[1]	STG	HSG	IRG	Sig[1]	STG	HSG	Sig[1]
WSC (g/kg DM)	167	200	208	**	195	215	215	*	161	170	159	NS	150	159	NS
CP (g/kg DM)	227	196	203	*	235	234	255	NS	256	262	283	***	249	255	NS
NDF (g/kg DM)	489	475	472	NS	393	376	360	NS	415	390	376	***	431	402	NS
ADF (g/kg DM)	235	235	232	NS	212	204	200	NS	211	205	198	*	247	219	NS
Milk Yield (kg/day)	20.9	20.9	21.7	NS	25.1	25.5	25.7	NS	9.6	11.3	12.0	NS	11.0	11.7	*
MS yield (kg/day)	1.72	1.7	1.76	NS	20.3	20.9	2.14	NS	0.89	1.03	1.04	NS	0.99	1.09	**

[1]Significance of treatment: NS; not significant, * P<0.05, **P<0.01, ***P<0.001

During the same experiments, milk urea nitrogen (MUN) concentration was measured for spring (2004 and 2005) and autumn (2006) (Cosgrove et al., 2007). There was no significant difference between grasses in spring, however MUN was lower in autumn for cows fed the HSG compared with the IRG and STG. High concentration of MUN denotes excess ammonia formed in the rumen, which suggests increased N urinary excretion (Jonker et al., 2002). The authors concluded that possible benefits in reducing N excretion can be attained by feeding dairy cows with HSG during autumn.

Two further experiments were conducted in NZ to test differences in spring and autumn using the same proportion of late and early lactation cows in both seasons, spring and autumn (Pacheco et al., 2009). Results presented in Table 1.6 are the mean of values from each treatment. Nitrogen intake and excretion were not measured directly in these grazing experiments but were calculated using indirect methods.

Table 1.6: Herbage chemical composition, estimated intakes of nitrogen (N) and its partition between urine and milk for STG: standard perennial ryegrass; cv. Impact (control); HSG: perennial high sugar grass; cv. AberDart and IRG: tetraploid Italian annual ryegrass; cv. Moata (Pacheco et al., 2009).

	Spring (2007)				**Autumn (2008)[1]**			
	STG	HSG	IRG	Sig[2]	STG	HSG	IRG	Sig[2]
Plant composition								
WSC (g/kg DM)	194	216	217	NA	144	160	139	NA
CP (g/kg DM)	184	190	178	NA	265	272	280	NA
NDF (g/kg DM)	451	433	437	NA	377	338	356	NA
N intake (g/day)	408	409	374	**	522	545	550	**
N output urine (g/day)	215	222	147	**	276	336	343	**
Proportion of N intake in urine[3]	0.53	0.54	0.39	NA	0.53	0.62	0.62	NA
N output milk (g/day)	109	110	106	*	101	107	107	NS
Proportion of N intake in milk[4]	0.27	0.27	0.28	NA	0.19	0.20	0.19	NA

[1] Data for period 1 and 3 only. Period 2 was not possible due to grass shortage
[2]Significance level: NA; not available, NS; not significant, * P<0.05, **P<0.01, ***P<0.001
[3] Calculated as N output in urine/ N intake
[4] Calculated as N output in milk/ N intake

All grass treatments contained less CP and more WSC and NDF in spring than in autumn. In spring, the greatest concentration of WSC was for the IRG treatment followed by HSG and the control. In autumn, HSG showed the greatest concentration of WSC. High sugar grass treatment had the lowest concentration of NDF in both seasons, although plant composition data were not tested statistically. Estimated N intakes were different between treatments and were higher in autumn than in spring. In spring, cows eating the IRG treatment had the lowest N intake and consequently excreted less N in urine. In autumn, cows in the IRG treatment had the highest levels of urinary N excretion coincident with the highest N intake.

1.5.3 The importance of the CP:WSC ratio

The hypothesis for the potential benefits of higher WSC concentration in grasses has been that higher WSC may correct the asynchrony between the supply of energy and protein in the rumen of animals fed fresh forage diets (Kingston-Smith & Theodorou, 2000). To test this, it is necessary to compare N use efficiency not only for different WSC concentrations but for a range of CP:WSC (or WSC:CP) ratios (Edwards et al., 2007; Pacheco et al., 2007). Edwards et al., (2007) plotted the data from the UK and Dutch experiments (Figure 1.6 A), showing reduced urinary N excretion with increasing WSC:CP ratio: especially 0.7 or above (equivalent to CP:WSC of 1.3 or below; Pacheco et al., 2007) markedly reduced the proportion of dietary N that was excreted in urine. However, to achieve a high WSC:CP (or a low CP:WSC) more sugar is required in high CP forages than in low CP herbage (Edwards et al., 2007). For example, a forage containing 125 g/kg of CP would require 188 g/kg of WSC to achieve a WSC:CP ratio of 1.5 and a typical NZ diet of fresh pasture with 250 g/kg DM of CP would require 376 g/kg of WSC to achieve the same ratio.

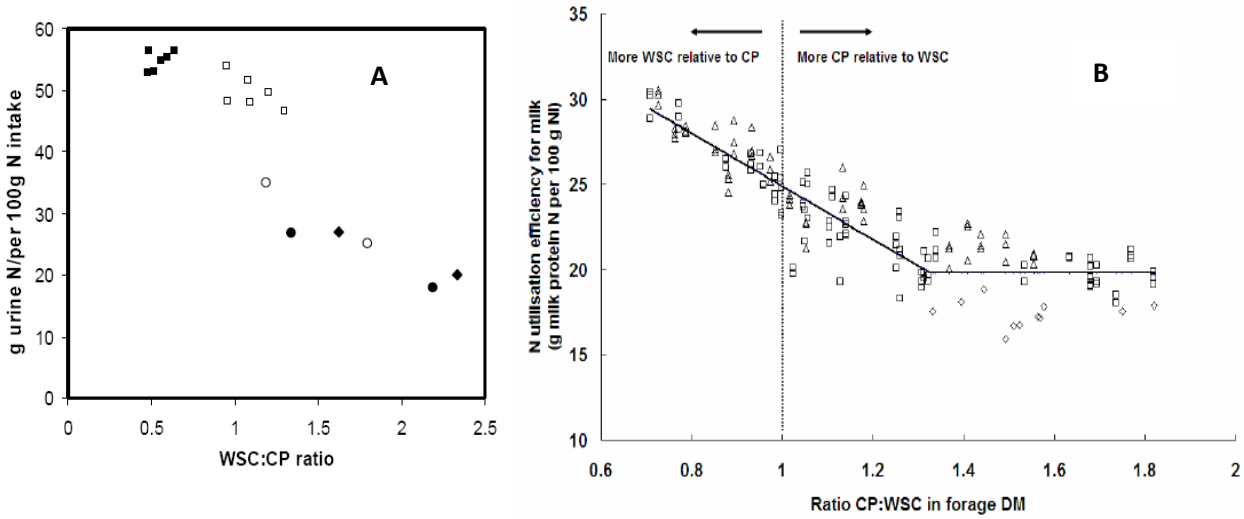

Figure 1.6: Relationship between nitrogen (N) urinary excretion (A) or efficiency of N utilisation for milk production (B) and different WSC:CP (or CP:WSC) ratios for a range of experiments in the United Kingdom, the Netherlands and New Zealand. From (A) Edwards et al., (2007) and (B) Pacheco et al., (2007).

Pacheco et al., (2007) plotted data from the experiments conducted in NZ during 2004, 2005 and 2006 (Figure 1.6 B). Figure 1.6 B shows the scatterplott of the estimated values for nitrogen utilisation efficiency for milk production (NUEm) against the CP:WSC ratio of the grass consumed by lactating cows. The breakpoint in this figure, CP:WSC=1.3, is equivalent to the value published by Edwards et al., (2007); WSC:CP = 0.7. From Figures 1.6 A and B it is evident that the benefits for the dairy industry in terms of increased milk N yield/100 g N consumed and reduced urinary N excretion are most likely to occur if ryegrasses can be produced with WSC:CP ratios of 0.7 or above.

Note that in Chapter 2 calculations will be done using the **CP:WSC** ratio.

1.6 Summary and Conclusion

- The NZ dairy industry is highly dependent on the utilisation of grazed perennial ryegrass (0.8): white clover (0.2) pastures as the main source of feed for dairy cows. Metabolisable energy intake is often the factor that limits milk production from cows grazing fresh forage diets while protein intake usually exceeds animals' requirements. Protein in fresh forages is highly degradable in the rumen, with large amounts of ammonia being produced. When energy, in the form WSC, is not sufficient or not available in synchrony with the rate of protein breakdown in the rumen, large amounts of ammonia are absorbed from the rumen.

- Excess ammonia, which cannot be captured for microbial protein synthesis by ruminal microorganisms, is absorbed from the rumen, detoxified to urea in the liver and excreted in urine, representing inefficient dietary N utilisation by grazing livestock. Excretion of large amounts of N into the environment contributes to ground and surface water pollution mainly through nitrate leaching and increase in nitrous oxide emissions, a potent greenhouse gas.

- A new cultivar of ryegrass (HSG), developed in the UK, was selected using traditional breeding techniques to contain higher concentrations of WSC. The development of HSG offers an opportunity to grow the dairy industry while at the same time minimise environmental impacts associated with N pollution. The potential benefits of HSG have been tested in Europe and NZ. Studies have been conducted to evaluate possible increases in milk and MS yields and reduction of N excreted in urine.

- Selection for the HSG trait generally tended to increase plant WSC concentration and decrease CP and NDF concentration in studies conducted in Europe and NZ, although the expression of the higher WSC trait showed a large variation among the experiments.

Greater expression of the trait would be useful in herbage with high CP concentration like those grazed in NZ, especially during autumn.

- In the studies conducted in Europe the most consistent result was reduced urinary N excretion as a proportion of N intake in cows fed the HSG diet. Milk yield from cows fed HSG increased only in one experiment in autumn. New Zealand studies showed increased milk and MS yield in cows grazing HSG only in autumn for one particular year (2007). Reductions in urinary N excretion were suggested for autumn 2006 but not for the other seasons analysed.

- New Zealand authors proposed that the ratio WSC:CP (or CP:WSC) is more important, in order to determine animal responses, than plant WSC concentrations alone. Increases in WSC:CP ratio above 0.7 (or decreases in the CP:WSC ratio below 1.3) were associated with reduced partition of ingested N to urine N and increased partition to milk N. This also suggests that to have a greater WSC:CP ratio (or smaller CP:WSC ratio) it is necessary to increase WSC concentration whilst decreasing CP concentration in the grass at the same time.

- It is necessary to conduct more field studies in NZ to confirm potential benefits of HSG and determine to what extent the trait is expressed and how animal responses can be improved. Pastures used in the studies reviewed here were sown as pure grass to avoid confounding effects of white clover on protein intake. However, grasses with elevated WSC should be examined in the presence of the legume because this mixture (perennial ryegrass + white clover) is the preferred combination used on NZ farms.

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CHAPTER 2

Milk production and nitrogen partitioning in dairy cows grazing standard and high sugar perennial ryegrass, with and without white clover, during spring and autumn

CHAPTER 2

Milk production and nitrogen partitioning in dairy cows grazing standard and high sugar perennial ryegrass, with and without white clover, during spring and autumn.

2.1 Introduction

In New Zealand (NZ), dairy cows are required to meet their nutritional needs from fresh grazed pasture, mostly perennial ryegrass and a small proportion of white clover. Grazed pasture provides a cheap but good source of quality feed (11.5-12 MJ ME/kg DM; Burke et al., 2002), which can support moderate levels of milk production (Burke et al., 2002; Kolver et al., 2002). Fresh temperate NZ pasture usually contains a higher concentration of protein (250-300 g/kg DM; Burke et al., 2002) than required by the animal (Burke et al., 2002; Holmes et al., 2002; Waghorn & Clark, 2004). This problem is often intensified by the application of high rates of nitrogen (N) fertilisers to boost pasture production (Valentine & Kemp, 2007), notably in autumn, which further increases herbage crude protein (CP) concentration. Moreover, in temperate grass-based systems the concentration of water soluble carbohydrates (WSC) is relatively low (100-150 g/kg DM; Burke et al., 2002) in comparison to the high concentrations of CP. This causes an imbalance between protein and energy supply in the rumen and reduces rumen microbial efficiency to capture ammonia degraded from protein in the rumen.

Excess dietary N has negative implications for both animal production and the environment. Nitrogen that is in excess to what microbes can utilise in the rumen is converted into ammonia in the rumen and excreted as urea in the urine, representing an extra energetic cost for the animal (Ulyatt, 1997). Furthermore, environmental pollution is associated with large amounts of N concentrated in urine patches which is immediately available for leaching or volatilisation (Haynes & Williams, 1993).

High WSC concentration in perennial ryegrass is a desirable characteristic for efficient animal production and thus an important trait targeted by grass breeders. A breeding program at the Institute of Grassland and Environmental Research; IGER (now Institute of Biological, Environmental and Rural Sciences; IBERS) in the United Kingdom, has been focused on increasing the content of WSC in perennial ryegrass for several years (Humphreys, 1989a, 1989b, 1989c) using conventional breeding techniques. Many grass breeding lines were evaluated and as a result of these studies the cultivar AberDart, one of several perennial ryegrass cultivars commonly known as a 'high sugar grass' (HSG) was first marketed in 2000 (Edwards et al., 2007).

The purpose of the present study was to investigate whether a HSG (cv. AberDart) represented a valuable alternative to use on NZ dairy farms. The studies reviewed in section 1.5 (Chapter 1) have considered animal performance on ryegrass as the sole pasture fed to the cows. However, NZ farmers utilise this grass in combination with white clover. Until now, no studies have investigated the responses of cows in terms of milk production and N partitioning within the cow when ryegrasses with higher content of WSC are combined with white clover. The present research, conducted in NZ, focuses on this subject, comparing the performance of cows grazing HSG pastures with those cows grazing standard NZ perennial ryegrass pastures, both with and without white clover, in spring and in autumn.

2.2 Materials and Methods

2.2.1 Experimental design and treatments

Two field experiments were carried out on Massey University N^{o} 4 Dairy farm, situated on the outskirts of Palmerston North (40^{o}19'S, 157^{o}37E), from November 14th 2008 to January 18th 2009 (Experiment 1; spring), and from April 15th 2009 to May 26th 2009 (Experiment 2; autumn). Sixty early-mid lactation Friesian cows, in groups of 15, were allocated to one of four treatments in Experiment 1 (spring). Experiment 2 (autumn) used 20 late-lactation Friesian cows allocated in groups of five because the amount of grass grown in each treatment was not enough to sustain 60 cows. Grass treatments were two diploid cultivars of perennial ryegrass chosen to differ in WSC content: *Lolium perenne*, cv. AberDart –a proposed 'high sugar grass' (HSG), and cv. Impact as a NZ standard control. Both cultivars were sown with or without white clover (cl); HSG+cl, control+cl, HSG and control.

Both experiments were conducted using a cross-over design with four periods. Each group of cows was randomly allocated to a different treatment (in Period 1) for a 10-day measurement period and then followed a pre-determined allocation sequence (Table 2.1). At the end of each period, groups of cows (n= 15 and n=5 for Experiment 1 and 2, respectively) were crossed over to each of the other treatments.

Table 2.1: Sequence of treatments for each group of cows for Experiment 1 and 2.

	Period 1	**Period 2**	**Period 3**	**Period 4**
Group1	control	control+cl	HSG	HSG+cl
Group2	HSG	control	HSG+cl	control+cl
Group 3	control+cl	HSG+cl	control	HSG
Group 4	HSG+cl	HSG	control+cl	control

Cows were offered the same daily DM allowance in all treatment, with a target DMI of 18 kg/day in Experiment 1 and 16 kg/day in Experiment 2. Samples of pasture offered were collected and analysed for chemical and botanical composition. Milk yields were automatically recorded at every milking, and milk samples for fat, protein, lactose and urea N concentration were collected

from each cow three times per period. Urine samples were collected at the end of each measurement period on two occasions to estimate urinary N excretion. Cows' LW and body condition score were recorded at the beginning and at the end of each period.

2.2.2 Pastures

A total area of 20.3 ha comprising nine paddocks was used in both experiments. In March 2008 six paddocks (12.7 ha) were sown with new perennial ryegrasses. Three additional paddocks (7.6 ha) previously sown in 2004 were also used for both experiments. One randomly-selected half of each paddock was sown with the control and the other half with HSG. The two varieties of ryegrass were endophyte-free. Treatment paddocks that contained white clover were sown at a rate of approximately 16 kg of ryegrass seed and 4 kg of clover seed per ha. Clover-free paddocks were sown at a rate of 20 kg ryegrass seed per ha, following full cultivation. Total areas for control, HSG, control+cl and HSG+cl were 6.4, 6.4, 3.5 and 4 ha, respectively. Some of these paddocks had previously been in turnips for late summer feed and others in perennial ryegrass. No irrigation was applied at any time on these paddocks. In September 2008 before starting Experiment 1, all experimental paddocks were fertilised with urea (46 %N; Ravensdown, NZ) at a rate of 80 kg/ha. During the autumn trial N fertiliser was applied in April to all paddocks at a rate of 80 kg /ha of urea or 100kg ha of Ammo31 (31 %N; Ravensdown, NZ). Table 2.2 shows the characteristics and management of the paddocks used for both experiments.

Table 2.2: Main characteristics of experimental paddocks during the experiments.

Paddock	Treatment	Ha	Sowing date	Fertiliser application
6	HSG+cl and control+cl	2	March 08	80kg/ha urea
8	HSG+cl and control+cl	2.6	March 08	80kg/ha urea
51	HSG+cl and control+cl	2.9	March 08	100kg/ha Ammo31
4	HSG and control	1.5	March 08	80kg/ha urea
12	HSG and control	2.1	March 08	100kg/ha Ammo31
39	HSG and control	2.7	April 04	80kg/ha urea
41	HSG and control	1.6	March 08	80kg/ha urea
78	HSG and control	3	April 04	100kg/ha Ammo31
95	HSG and control	1.9	April 04	80kg/ha urea

2.2.3 Pasture allocation and grazing management

Experimental paddocks were rotationally grazed using semi-permanent electric fences. During each measurement period, for both experiments, groups of cows were offered fresh daily allowance of grass at 8 AM and 4 PM, immediately after milking. The target pre and post grazing herbage mass was 2,600 kg DM/ha and 1,600 kg DM/ha allowing for target intakes of 18 kg DM/cow/day and 16 kg DM/cow/day for spring and autumn, respectively. The area offered each day for each group, was calculated as:

$$\mathrm{BA} = \frac{n \times \mathrm{DMI}}{\mathrm{PPM\text{-}RPM}} \quad (1)$$

Where BA is break area in ha, *n* the number of cows, DMI is the target dry matter intake in kg/cow/day (18 kg DM in spring and 16 kg DM in autumn), PPM is the pre grazing pasture mass and RPM the desired residual pasture mass, both in kg DM/ha determined using an electronic rising plate meter (Farmworks Precision Farming Systems, Feilding, New Zealand) calibrated in previous experiments (Cosgrove et al., 2007; equation 1B).

$$\text{Herbage mass (kg DM/ha)} = 158 \times \text{herbage height (cm)} + 200 \quad (1B)$$

The values recorded with the plate meter were the average of approximately 50 readings taken randomly throughout the area allocated for grazing. Of the total area for daily grazing, 40% was offered following the morning milking and 60% following the afternoon milking to match the natural grazing behavior (and hence DMI) of cows as closely as possible (Cosgrove et al., 2006).

2.2.4 Animals

In spring, 60 Friesian cows were selected from the main herd and allocated to one of four treatments in groups of 15 cows each. Each group was balanced for age, days in milk and current production based on the most recent herd test data. During Experiment 1 eleven cows had to be replaced due to lameness and mastitis. These conditions were not associated with any of the treatments. Each animal was replaced by a matching cow from the main herd in terms of age,

days in milk and current production. Experiment 1 was interrupted on two occasions (one week between period 2 and period 3 and seventeen days between period 3 and 4) due to a shortage of experimental grass. On these occasions cows were kept on a sole grass diet on the farm. In autumn, the initial number of cows used was 40 (allocated in groups of ten). The first period was conducted using 10 cows per treatment, however for periods two, three and four it was not possible to sustain 40 cows due to very low pasture growth rates and only 5 cows in each group could complete the trial. Table 2.3 summarises cows' descriptors for each season.

Table 2.3: Mean (± SD) parity (number of lactations), liveweight (kg), body condition score (BCS; 10-point scale), days in milk, milk yield (kg/day), and number of cows (n) used for Experiment 1 and 2.

Experiment	Season	n	Parity	Liveweight	BCS	Days in milk	Milk yield
1	Spring	60	2.7 ± 1.5	481 ± 49	3.9 ± 0.5	85 ± 16	25.6 ± 3.8
2	Autumn	20	2.4 ± 1.0	464 ± 41	3.9 ± 0.5	233 ± 20	15.3 ± 3.4

2.2.5 Pasture measurements and pasture sampling

Samples of pasture offered to the cows were collected from the allocated breaks at 8 AM and 4 PM to account for diurnal differences, especially WSC, three times per period. Samples were hand- plucked from ten sites around each allocated break, to represent herbage being consumed by the cows in each grass treatment.

Pre and post grazing pasture mass was measured before and after cows grazed each allocated break, by cutting five (pre grazing) or ten (post grazing) sample quadrants (0.25 m^2) per break (PM and the subsequent AM) twice per period in all treatments. Cuts were made to soil level and samples were washed and dried overnight for 24 hours in a forced-air oven at 100^oC. Five representative samples of pre grazing pasture cuts were pooled for dissection into ryegrass, white clover, other grasses, dead matter and weeds. The results obtained with this pasture mass methodology were not satisfactory for calculating estimates of daily feed intake and this was not repeated in Experiment 2. Pasture botanical composition in autumn was estimated by cutting 10

quadrants (0.25 m^2) at soil level, every time cows shifted to a new paddock for all treatments. The samples obtained for each paddock were pooled for dissection as explained earlier.

To estimate pasture intake, in both experiments, pre and post grazing herbage mass was measured before and after grazing each break daily for all treatments using the calibrated rising plate meter. Feed intake was estimated as

$$\mathrm{DMI} = \frac{\mathrm{PPM\text{-}RPM}}{n} \times \mathrm{BA} \quad (2)$$

Where DMI is dry matter intake in kg /cow/day, *n* the number of cows, PPM is the pre grazing pasture mass and RPM the residual pasture mass, both in kg DM/ha determined with the rising plate meter, and BA is the offered break area in ha. This methodology only provides an average estimate of DMI for each treatment. Therefore individual intake was estimated using individual milk production and composition, and ME content of the diet offered (equations 5 and 6). Average estimated intakes for each treatment using both methodologies were similar. However, DMI results presented in this chapter and estimates of N partitioning were calculated using equations 5 and 6.

2.2.6 Animal measurements

2.2.6.1 Milk yield and composition

Milk yield (kg/cow) was recorded at each milking using an automated system (Alpro, De-Laval). Milk samples for fat, protein, lactose and urea N concentration were collected three times per period (coincident with pasture sampling) from proportional in-line samplers at a PM and the following AM milking. Milk samples were immediately refrigerated at 4^{o}C until processing in the lab. Daily MS yield (fat plus protein) was calculated using the fat and protein and the milk yields measured on the days of sample collection.

2.2.6.2 Urine

Spot urine samples were collected from each cow at the end of each period. Samples were collected on two consecutive days: at a PM and the following AM sampling (coincident with milk and pasture sampling) on two occasions during each period, in order to estimate urinary N excretion. A stimulatory massage was applied to the minority of cows that did not urinate voluntarily. A representative sample of mid-stream urination was collected in a plastic container and immediately placed in ice until processing in the lab.

2.2.6.3 Liveweight and body condition score

Liveweight and BCS were measured at the beginning and at the end of each period. Cows were individually weighed using a farm scale (Tru-Test, Auckland, New Zealand) after a morning milking. Body condition score was visually determined at the same time using the 10-point scale, in which 1 is emaciated and 10 is obese (Roche et al., 2004). Body condition score was always assessed by the same person.

2.2.7 Laboratory methods

2.2.7.1 Pasture

Forage samples were immediately frozen in liquid N after collection in the field, stored frozen (-20^{o}C) and subsequently freeze-dried (W.G.G Cuddon LTD, Blenheim, New Zealand) and ground to pass a 1 mm size sieve. They were analysed using near infrared reflectance spectroscopy (NIRS; calibrated for high sugar grasses, FeedTech, AgResearch Grasslands, Palmerston North, New Zealand) to estimate WSC, CP, NDF and ADF, OMD and ME. Dry matter content of the sample was determined from the sample fresh weight and the dry weight followed freeze-drying. For the two treatments that contained clover, the fresh sample was dissected into grass and clover, weighed and analysed separately.

2.2.7.2 Milk

In the lab, 30 ml of each milk sample (3 PM and 3 AM samples per period per cow) was transferred to a vial which contained 150 µl of 0.04% bronopol (2-bromo-2-nitropropane-1.3-diol) to preserve milk from microbial spoilage. Vials were refrigerated until they were analysed for fat, protein and lactose composition (at the end of each period) using a FT 6000 Fourier Transform infrared analyser (TestLink, Hamilton, New Zealand). The rest of the milk was kept refrigerated to estimate milk urea concentration. At the end of each period, the remainder of the 6 milk samples (3 PM and 3 AM samples per period per cow) was pooled together, mixed gently and an aliquot of 250 ml was kept in a labeled container to be frozen (-20^{o}C) for skimming at a later time, while the rest of the sample was discarded. The pooled whole milk samples were defrosted and centrifuged to separate fat on the Sorvall RG (H54 rotor 7500rpm) for 30 minutes at 4^{o}C. Skim milk samples were treated with a sodium acetate:acetic acid buffer to precipitate casein. Urea was determined in the milk supernatant by the urease-UV procedure, using a Flexor E clinical chemistry analyser (Vital Scientific, Dieren, The Netherlands) and a commercial diagnostic kit for urea (Roche Diagnostic NZ Ltd.) according to the manufacturer's recommendations. Milk urea content was multiplied by 2 to correct for N content in urea.

2.2.7.3 Urine

Urine collected from each cow was analysed for total N and creatinine content. Using a pipette, 3.5 ml of urine was placed into a vial for creatinine analysis and 7 ml pipetted into a bottle for total N analysis. This was repeated after each of the urine sampling occasions in all periods. All bottles and vials were stored refrigerated at 4^{o}C until the end of each period. After the last sampling in the period, the creatinine samples from each cow were gently mixed together and 1 ml of urine was sent to the lab for analysis. Creatinine in urine was determined by a spectrophotometric assay using a compensated Jaffe reaction in a Hitachi 902 automatic analyser (NZ Veterinary Pathology, Palmerston North, New Zealand). Bottles that contained urine for total

N analysis were weighed and each sample was then acidified (6 ml of 50% chloridric acid; HCl) to reduce the urine pH to less than 2.0. Bottles were weighed again to determine the weight of the added acid. Samples were analysed for total N concentrations using the combustion method in a Carlo Erba Nitrogen Analyser (Carlo Erba, Italy).

2.2.8 Calculations and statistical analysis

Urinary N excretion was calculated using urine total N concentration and creatinine concentration and average LW of cows in each period. The urinary N excretion was calculated as:

$$\text{UN excretion} = \frac{21.9 \text{ x BW}}{\text{Urinary creatinine(mg/kg)}} \text{ x N} \quad (3)$$

Where UN excretion is urinary nitrogen excretion in g/day, 21.9 (mg/kg) times body weight (BW) is the creatinine excretion factor calibrated from an experiment in which total urine collection was performed in lactating pasture-fed cows (n= 15; Pacheco, unpublished) and N is the nitrogen concentration in urine in g/kg.

Nitrogen secreted in milk in g/day was calculated as:

$$\text{Milk N} = \frac{\text{milk protein yield (g/day)}}{6.38} \quad (4)$$

Dry matter intake per cow was calculated from the energy requirement of the cows (AFRC, 1993) using the average LW of each cow in each period, the average measured milk production and composition (fat, protein and lactose) of each cow and the average ME content of pasture:

$$\text{Milk E req} = \frac{(0.0384 \text{ x F} + 0.0223 \text{ P} + 0.0199 \text{ L - } 0.108) \text{ x milk yield}}{0.65} \quad (5)$$

Where Milk E req is the metabolisable energy required for milk production based on fat (F), protein (P) and lactose (L) concentration (g/kg), and milk yield (kg/day) as an average per cow for

the two sampling days at the end of each measurement period; and 0.65 is the energy efficiency to synthesise milk from ME (Holmes et al., 2002).

$$\text{DMI} = \frac{\text{Milk E req.} + (\text{ average LW}^{0.75} \text{ x } 0.60)}{\text{ME}} \quad (6)$$

Dry matter intake (DMI) is estimated as the sum of the energy required for milk production and the energy required for maintenance, assuming no LW change divided by the average ME concentration of the pasture on the two days of sampling coincident with milk and urine sampling.

Nitrogen intake was calculated as:

$$\text{NI} = \frac{\text{DMI x CP}}{6.25} \quad (7)$$

Where NI is nitrogen intake in g/day, DMI is the estimated DMI per cow in kg/day (equation 6) and CP is the crude protein content of forage in g/kg DM (average value from the two sampling days at the end of each period).

2.2.8.1 Statistical analysis

All data were analysed using the Statistical Analysis System package (SAS, 2003; version 9.1). Analysis of variance for plant variables were performed with the MIXED procedure. The linear model included the fixed effects of treatments, period and their interaction, and the random effect of paddock. Paddock was considered the experimental unit and repeated measures on the same paddock were considered with homogenous variance. Means and standard errors for each variable for each treatment were obtained and probabilities associated to multiple treatment comparisons were obtained using the Tukey's test as an option within the MIXED procedure.

Animal variables were analysed using the MIXED procedure. The linear model included the fixed effects of treatment and the day of the experiment (within each period) and the random effect of period, group of cows and the interaction between period, group of cows and treatment.

Using the Akaike's information criterion, a compound symmetry was determined as the most appropriate residual covariance structure for repeated measures on the same cow over days within periods.

Main effects of inclusion of white clover, ryegrass cultivar and their interaction upon milk and MS yield, pasture chemical composition and nitrogen partition were analysed using the CONTRAST statement in MIXED procedure.

Estimates of the regression lines (intercept and slopes) of change in N output on N intake and change in N utilisation efficiency on CP:WSC ratio were obtained using the GLM procedure. For both, animal and pasture related variables, differences were declared significant for a probability value lower than 0.05.

2.3 Results

2.3.1 Pasture botanical composition and herbage mass

Botanical composition and herbage mass (pre and post grazing) are shown in Table 2.4 for both experiments. There was no difference among treatments for pre and post grazing herbage mass in Experiment 1. However, in Experiment 2 pre grazing herbage mass differed ($P<0.05$) among treatments, with HSG+cl having the lowest pasture cover (2394 kg DM/ha) while the control treatment had the highest average pre grazing cover (2729 kg DM/ha). Post grazing herbage mass did not differ among treatments for Experiment 2.

The proportion of white clover for the control+cl and the HSG+cl was 7.2 % and 9.2%, respectively for Experiment 1, and 1.8% and 2 % for Experiment 2. In the treatment paddocks containing white clover, the proportion of other grasses was lower than for no clover treatments, in both experiments, while the proportion of sown perennial ryegrass tended to be higher. Pasture dead matter content was approximately 20 % in Experiment 1 (spring) and 30 % in Experiment 2 (autumn), with the lowest values in the HSG+cl treatment in both experiments.

Table 2.4: Herbage mass and botanical composition for control (cv. Impact) and for high sugar (HSG; cv. AberDart) perennial ryegrass sown with or without white clover, grazed by lactating dairy cows in Experiment 1 (spring) and Experiment 2 (autumn).

	control		HSG		Pooled SEM	P value
	No clover	clover	No clover	clover		
Experiment 1						
Herbage mass (kg DM/ha)						
Pre grazing	2880	2993	2701	2807	128.2	0.420
Post grazing	1812	1923	1804	1782	49.3	0.253
Botanical composition (%DM)[1]						
Ryegrass	57.7	64.2	65.2	64.1	5.30	0.663
White clover	-	7.2	-	9.2	1.02	0.212
Dead matter	24.4	20.2	20.5	15.3	2.77	0.209
Other grasses	18.0	10.2	14.9	10.7	7.30	0.847
Weed species	1.3	1.5	1.7	0.7	0.73	0.803
Experiment 2						
Herbage mass (kg DM/ha)						
Pre grazing	2729[a]	2661[ab]	2499[b]	2394[b]	87.2	0.032
Post grazing	1604	1619	1682	1573	59.1	0.585
Botanical composition (%DM)[1]						
Ryegrass	50.5	57.5	54.3	69.3	6.36	0.252
White clover	-	1.8	-	2.0	0.43	0.793
Dead matter	36.9	36.4	34.7	27.9	3.88	0.407
Other grasses	9.8	3.1	10.5	0.4	5.26	0.480
Weed species	2.8	1.1	0.6	0.4	1.19	0.462

[a & b] For the same row, means with different script differ significantly ($P < 0.05$)

[1] Obtained as a result of quadrant cuts

2.3.2 Pasture chemical composition

Herbage chemical composition in diets selected by the cows is shown in Table 2.5. Concentrations of WSC were lower in autumn than in spring, whilst concentrations of CP were much higher in autumn herbage. Organic matter digestibility and ME concentration of herbage were similar in both seasons. Concentration of CP, WSC and DM content was similar for all treatments in Experiment 1. However, for all other parameters there were significant differences among treatments. Inclusion of white clover in the diets was associated with a reduced herbage NDF ($P<0.001$) and ADF ($P<0.01$), increased OMD ($P<0.01$) and higher ME concentration ($P<0.01$) in Experiment 1 (Table 2.6). The HSG diets had lower concentration of NDF than control diets

(P<0.05) in Experiment 1 and tended to decrease ADF concentration (P=0.09) and to increase ME (P=0.07) compared with the control diets. There were significant interactions (P<0.05) between the ryegrass cultivar and the presence of white clover for NDF and ME, explained by NDF being lowest (417g/kg DM) and ME highest (12.6 MJ ME/kg DM) in the HSG+cl treatment. In Experiment 2, concentration of NDF was also the lowest (P<0.05) for the treatments containing white clover. High sugar grass diets showed lower concentration of NDF (P<0.001) and ADF (P<0.01) than control grass diets for Experiment 2 and higher concentrations of WSC (P<0.01). There were significant ryegrass cultivar x white clover interactions in Experiment 2 for WSC and ADF (P<0.05) and for NDF (P<0.001), explained by higher values of WSC and lower fibre concentration in the HSG+cl treatment (Table 2.6).

Table 2.5: Herbage chemical composition for control (cv. Impact) and for high sugar (HSG; cv. AberDart) perennial ryegrass sown with or without clover in Experiment 1 (spring) and Experiment 2 (autumn).

	control		HSG		Pooled SEM	P value
	No clover	clover	No clover	clover		
Experiment 1						
Chemical composition (g/kg DM)[1]						
DM	227	230	239	213	0.7	0.136
OMD	786^{a}	808^{ab}	786^{a}	837^{b}	11.4	0.018
CP	156	162	150	179	9.2	0.184
WSC	213	224	238	230	9.7	0.266
NDF	476^{a}	445^{b}	461^{ab}	417^{c}	8.0	0.0006
ADF	287^{a}	272^{ab}	282^{a}	258^{b}	5.2	0.0057
ME(MJ/kg DM)	12.0^{a}	12.2^{a}	12.0^{a}	12.6^{b}	0.12	0.010
Experiment 2						
Chemical composition (g/kg DM)[1]						
DM	186	179	199	197	1.0	0.442
OMD	800	816	780	824	12.8	0.477
CP	226	234	244	250	8.2	0.186
WSC	96^{a}	99^{ac}	111^{bc}	115^{b}	4.7	0.028
NDF	458^{a}	447^{a}	415^{b}	396^{b}	7.0	0.0001
ADF	249^{a}	248^{a}	238^{ab}	233^{b}	4.3	0.049
ME (MJ/kg DM)	11.7	12.0	11.8	12.1	0.20	0.512

[a, b & c] For the same row, means with the same letter do not differ significantly (P > 0.05)

[1] Obtained as a result of 'handpluck' samples

Table 2.6: Probability values (P value) of main effects of white clover presence , ryegrass cultivar and their interaction, on herbage chemical composition for Experiment 1 (spring) and 2 (autumn).

	White clover effect P value	Ryegrass cultivar effect P value	Ryegrass cultivar x white Clover effect P value
Experiment 1			
ADF	0.002	0.096	0.084
NDF	0.0003	0.017	0.030
OMD	0.006	0.219	0.099
ME	0.005	0.075	0.038
Experiment 2			
ADF	0.515	0.004	0.024
NDF	0.048	<0.0001	<0.0001
WSC	0.473	0.008	0.033

2.3.3 Milk and milk solids yields, milk composition and milk urea nitrogen

The inclusion of white clover in the diets in Experiment 1 significantly increased the yields of milk, MS, fat, protein and lactose ($P<0.01$) (Table 2.7). There was also a significant ryegrass cultivar x white clover interaction ($P<0.05$), explained by these milk yields being highest in cows grazing the HSG+cl treatment (21.6 kg/day; Table 2.8).

Table 2.7: Probability values (P value) of main effects of white clover presence , ryegrass cultivar and their interaction, on milk and milk solids yields and milk constituents for Experiment 1 (spring).

	White clover effect P value	Ryegrass cultivar effect P value	Ryegrass cultivar x white Clover effect P value
Experiment 1			
Milk yield	0.003	0.128	0.034
Milk solids yield	0.003	0.241	0.065
Fat yield	0.006	0.493	0.179
Protein yield	0.001	0.065	0.013
Lactose yield	0.001	0.088	0.014

Milk yield and milk composition from cows grazing all treatments in both experiments are shown in Table 2.8. There were no effects of the treatments applied upon milk yields or composition in Experiment 2. Milk urea N concentrations were similar among treatments in Experiment 1 and tended to be higher (P=0.068) for the HSG+cl treatment in Experiment 2.

Table 2.8: Milk and milk solids (MS) yields, milk composition and milk urea nitrogen (MUN) concentration from lactating dairy cows offered fresh control (cv. Impact) or high sugar (HSG; cv. AberDart) perennial ryegrass sown with or without white clover, in Experiment 1 (spring) and Experiment 2 (autumn).

	control		HSG		Pooled SEM	P value
	No clover	clover	No clover	clover		
Experiment 1						
Milk yield(kg/day)	19.4[a]	20.3[ab]	19.3[a]	21.6[b]	0.75	0.009
MS(kg/day)	1.46[a]	1.56[ab]	1.44[a]	1.67[b]	0.057	0.011
Milk composition (%)						
Fat	4.4	4.5	4.4	4.3	0.09	0.690
Protein	3.4	3.4	3.4	3.5	0.05	0.537
Lactose	4.9	4.9	4.9	4.9	0.04	0.155
Milk constituents yield(kg/day)						
Fat	0.83[a]	0.88[ab]	0.81[a]	0.93[b]	0.035	0.024
Protein	0.64[a]	0.68[a]	0.63[a]	0.74[b]	0.023	0.003
Lactose	0.92[a]	0.97[a]	0.91[a]	1.05[b]	0.040	0.003
MUN (mmol/l)[1]	5.9	7.0	5.4	6.6	0.63	0.333
Experiment 2						
Milk yield(kg/day)	11.0	11.3	11.2	11.9	0.68	0.687
MS (kg/day)	1.03	1.05	1.06	1.13	0.051	0.417
Milk composition (%)						
Fat	5.3	5.3	5.4	5.2	0.16	0.567
Protein	4.2	4.2	4.3	4.2	0.14	0.861
Lactose	4.8	4.8	4.8	4.8	0.05	0.981
Milk constituents yield(kg/day)						
Fat	0.57	0.58	0.59	0.61	0.029	0.717
Protein	0.45	0.46	0.47	0.50	0.022	0.404
Lactose	0.52	0.53	0.53	0.57	0.033	0.514
MUN (mmol/l)[1]	14.3	16.1	14.5	16.9	0.642	0.068

[a & b] For the same row, means with the same script do not differ significantly (P > 0.05)

[1] Calculated as the urea content in milk multiplied by 2 to correct for N content of urea

2.3.4 Nitrogen partitioning within the cow

Despite DMI being similar for all treatments in both experiments, N intake in Experiment 1 increased by the inclusion of white clover (P<0.01) and by HSG (ryegrass cultivar effect) in

Experiment 2 (P<0.05) (Table 2.9). Calculated N partitioning is shown in Table 2.10 for both experiments. On average, cows in Experiment 2 (autumn) had almost 19% more N intake than cows in Experiment 1 (spring) due to the higher concentration of CP in the autumn herbage. No differences among treatments were found in urinary N excretion (g/day) in spring or autumn. The proportion of N intake excreted in urine or secreted in milk was similar for all treatments in both experiments, with the lowest proportions found in spring. Nitrogen output in milk (g/day) in Experiment 1 was increased by the presence of white clover (P<0.001), with a significant ryegrass cultivar x clover interaction (P<0.05) explained by the highest values for the HSG+cl treatment (Table 2.9).

Table 2.9: Probability values (P value) of main effects of white clover presence, ryegrass cultivar and their interaction, on nitrogen (N) intake in Experiment 1(spring) and 2 (autumn); and milk N output in Experiment 1.

	White clover effect P value	Ryegrass cultivar effect P value	Ryegrass cultivar x white Clover effect P value
Experiment 1			
N intake	0.006	0.732	0.175
Milk N output	0.0004	0.179	0.011
Experiment 2			
N intake	0.143	0.022	0.044

Table 2.10: Estimated dry matter (DMI) and nitrogen (N) intakes and estimated N partition between urine and milk for lactating dairy cows offered control (cv. Impact) or high sugar (HSG; cv. AberDart) perennial ryegrass sown with or without white clover, in Experiment 1 (spring) and Experiment 2 (autumn).

	control		HSG		Pooled SEM	P value
	No clover	clover	No clover	clover		
Experiment 1						
DMI (kg /day)[1]	16.9	16.9	16.4	17.0	0.33	0.384
N intake (g/day)	437[ab]	467[bc]	416[a]	499[c]	40.9	0.025
N output						
Urine(g/day)	139	159	125	151	20.6	0.546
Proportion of N intake	0.32	0.34	0.30	0.31	0.033	0.813
Milk(g/day)	101[a]	108[b]	98[a]	118[c]	3.5	0.0009
Proportion of N intake	0.24	0.23	0.25	0.24	0.028	0.474
Experiment 2						
DMI(kg /day)[1]	14.1	14.3	14.2	14.2	0.45	0.971
N intake (g/day)	516[a]	529[ab]	545[b]	571[c]	13.7	0.002
N output						
Urine(g/day)	274	309	301	328	44.3	0.577
Proportion of N intake	0.53	0.58	0.55	0.57	0.079	0.868
Milk(g/day)	69	72	71	77	4.4	0.370
Proportion of N intake	0.13	0.13	0.13	0.13	0.006	0.898

[a, b & c] For the same row, means with the same script do not differ significantly (P > 0.05).
[1] Estimated using equations 5 and 6.

2.3.5 Interrelationships involving the partition of nitrogen excretion

Nitrogen intake partitioning between milk and urine is shown in Figure 2.1 A and B. As N intake (g/day) increased, there was a linear increase in the amount of N excreted in urine (g/day) (equation 8) but no relationship was found between N intake (g/day) and N secreted in milk (g/day) (equation 9).

N Urine = -119.07 + 0.652 x **N intake**; $\mathbf{R^2}$**=** 0.35; **S.E.** = 25.36, 0.05;

P = <0.0001, <0.0001 (8)

N Milk = 99.57 – 0.0083 x **N intake;** R^2**=** 0.001; **S.E.** = 6.94, 0.014;

P = <0.0001, 0.567 (9)

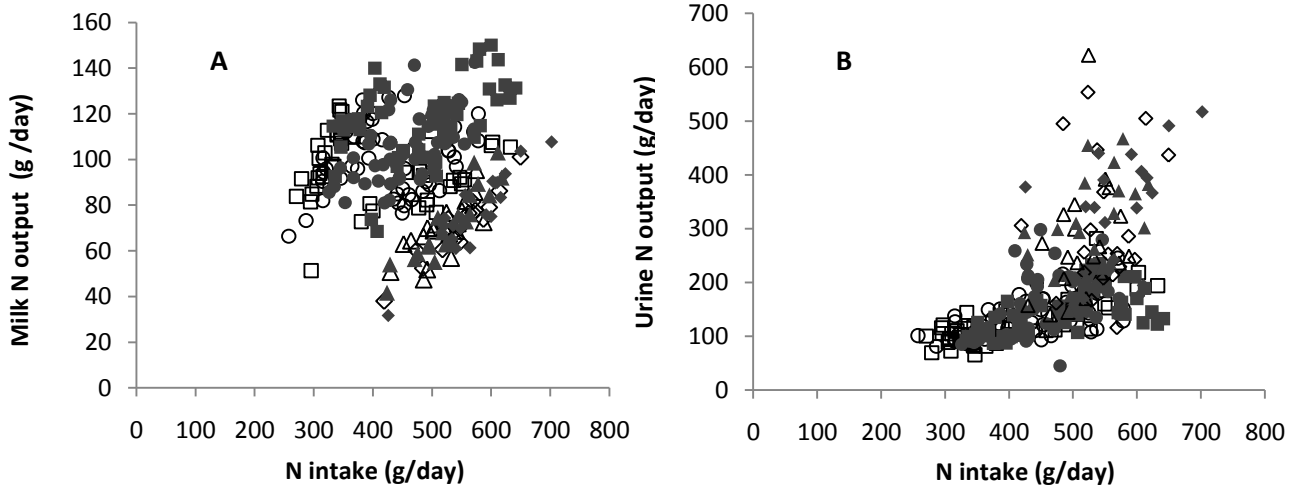

Figure 2.1: Partition of dietary nitrogen (N) intake between milk (A) and urine (B) in control (○), control+cl (●), HSG (□), HSG+cl (■), in Experiment 1 (spring) and control (Δ) control+cl (▲) , HSG (◊), HSG+cl (♦) in Experiment 2 (autumn). Each observation corresponds to average values of N intake and N output estimated from the last two sampling days in each experimental period for each cow.

Nitrogen utilisation efficiency for milk (NUEm; g milk N/100g N intake) and urine (NUEu; g urine N/100 g N intake) was plotted against the CP:WSC ratio of the herbage consumed (Figure 2.2 A and B). For NUEm two linear regression equations were fitted (equation 10 and 11), one for each experiment, whilst only one linear regression equation (equation 12) was fitted for NUEu data.

NUEm (spring) = 38.678 – 18.518 x **CP:WSC**; R^2**=** 0.92; **S.E.** = 1.227, 1.479;

P = <0.0001, <0.0001 (10)

NUEm (autumn) = 15.563 – 1.003 x **CP:WSC**; R^2**=** 0.10**;** **S.E.**= 1.869, 0.804;

P = <0.0001, 0.233 (11)

NUEu = 20.420 + 15.276 x **CP:WSC**; **R^2=** 0.60; **S.E.**= 4.010, 2.298;

P = <0.0001, <0.0001 **(**12)

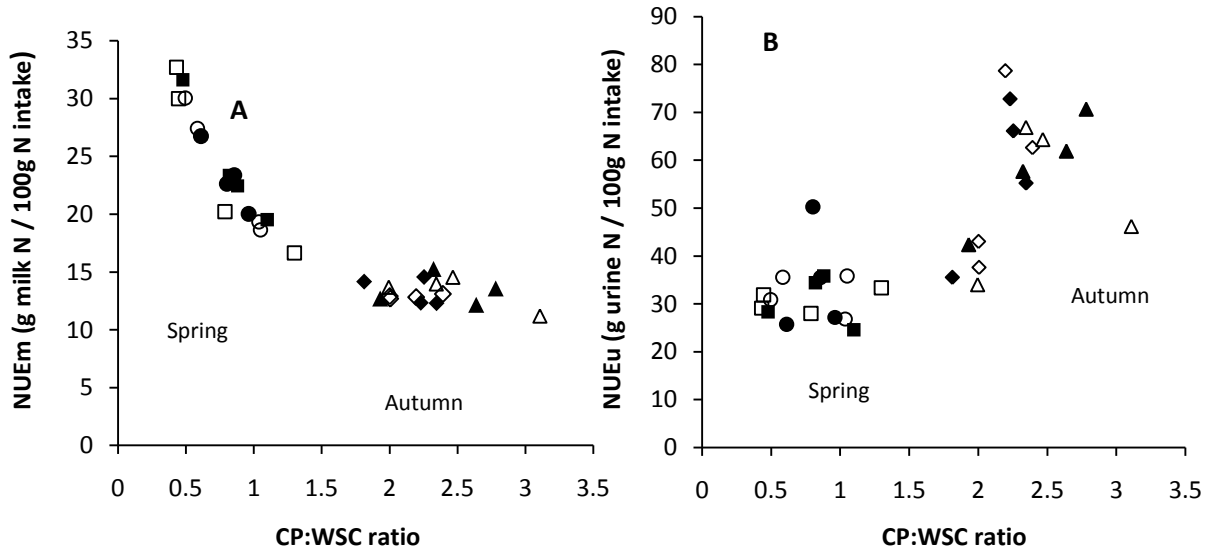

Figure 2.2: Estimated values of nitrogen utilisation efficiency for milk production (NUEm; A) and urine excretion (NUEu; B) against the ratio CP:WSC of the grass consumed in control (○), control+cl (●),HSG (□), HSG+cl (■), in Experiment 1 (spring) and control (Δ) control+cl (▲), HSG (◊), HSG+cl (♦) in Experiment 2 (autumn). Each observation represents the mean of a group of animals grazing one of the four treatments in each experimental period.

As the amount of WSC increased in the diet relative to the amount of CP (thus a lower CP:WSC) there was a significant increase in the efficiency of N utilisation for milk production (g milk N/100g N intake) in spring (equation 10). In autumn, NUEm was not related to CP:WSC (equation 11) (Figure 2.2 A). Nitrogen utilisation efficiency for milk production was higher in spring (lower ratio CP:WSC) than it was in autumn. The point of intersection of the two linear regression equations corresponds to a CP:WSC ratio of 1.32 and the NUEm for that breakpoint is 14 g milk N per 100 g N intake. The proportion of N intake appearing as urinary N excretion increased with increasing herbage CP:WSC ratio (Figure 2.2 B; equation 12).

2.4 Discussion

The objective of this study was to evaluate whether the use of HSG sown with or without white clover could be used on NZ dairy farms to increase milk production and/or decrease the amount of N excreted in urine by lactating cows. It has been postulated that higher levels of WSC, as in the HSG cultivar, would provide energy for rumen microbes to capture more N released from herbage protein degradation, preventing excessive loss of ammonia from the rumen and increasing AA absorption from the small intestine (Kingston-Smith & Theodorou, 2000).

Increases in milk production may be expected as a consequence of improving N utilisation in the rumen when feeding HSG (Miller et al., 2001); there is also evidence that reducing the amount of N excreted in urine is feasible (Miller et al., 2000, 2001; Moorby et al., 2006). It has been hypothesised that the use of ryegrasses with higher levels of WSC may correct the imbalance between energy and protein supply in the rumen of cows fed fresh forages and this may lead to a more sustainable way of farming grazing ruminants (Kebreab et al., 2002; Kebreab et al., 2004).

In contrast to UK data (Miller et al., 2000, 2001; Moorby et al., 2006) and other European data (Lee et al., 2002), use of HSG in NZ in the present study produced either no increase in herbage WSC concentration (Experiment 1) or only a small increase in Experiment 2. Data presented in Figure 2.2 suggest that the CP:WSC ratio in perennial ryegrass may be important in the partitioning of absorbed N into milk or urine. This and effects upon sward composition will be further examined in this discussion.

The lack of increase in milk N secretion, coupled with the large increase in urine N secretion as N intake increased (Figure 2.1), confirms that N intake was not limiting milk production in cows fed fresh forage diets in this study, as also shown in modeling studies which produced similar results (Kebreab et al., 2002). This gives confidence in the N transactions calculated in the present study, which were done using indirect methods. Also, these indirect methods were applied to all four treatments (in both experiments), so any relative differences identified should be valid.

As shown in Table 2.5, a consistent difference among treatments in the concentration of plant fibre was found in this study, where the HSG+cl (Experiment 1-spring) and the HSG treatments both with and without clover (Experiment 2-autumn) showed the lowest concentration of NDF. There was also a tendency for the HSG+cl treatment to show the lowest dead matter content in both experiments, although it was not statistically significant. Previous studies that compared cultivars with elevated concentrations of WSC suggested that increased levels of WSC concentrations in herbage are compensated by a reduction in CP and/or NDF concentrations (Lee et al., 2002; Miller 2001; Moorby et al., 2006; Vibart et al., 2009). In our study, neither the concentration of WSC nor CP were significantly affected by HSG in Experiment 1 and only small increases (15 g/kg DM) were observed for WSC concentration in HSG in Experiment 2. Lower NDF concentration and reduced dead matter content in the HSG+cl sward in Experiment 1 was associated with an increase in the OMD and ME concentration in that treatment.

Using cows in late lactation (comparable to Experiment 2) in the UK, Miller et al., (2001) reported increases in milk yield (2.7 kg/cow) when cows were fed HSG (cv. AberDove) indoors compared with a control grass, but no significant differences were found when using cows in early lactation (comparable to Experiment 1)(Moorby et al., 2006). In the UK late lactation study the WSC concentration in the HSG was elevated by 40 g/kg DM and the increase in WSC was associated with a reduction in plant CP and NDF concentration (Miller et al., 2001). Similarly, Cosgrove et al., (2007) reported small increases in milk yield (0.7 kg/cow) and MS yields (0.1 kg MS/cow) when cows were grazing HSG(cv. AberDart) in autumn (late lactation), but the concentration of WSC of the HSG was similar to the control. In both studies, the positive effects on milk production were associated with lower structural fibre concentration in the HSG diet, which increased OMD and this led to a higher digestible DMI although the total DMI did not change (Miller et al 2001).

There was a trend for increasing CP content in the clover treatments in both Experiment 1 and 2. Each treatment contained more CP and less WSC in autumn compared with spring, which is an

expected pattern in temperate dairy pasture (Burke et al., 2002: Litherland & Lambert, 2007). The lower dead matter content and low fibre concentration in the HSG+cl treatments was associated with increased milk production in Experiment 1 (early-mid lactation) and Experiment 2 (late lactation), although in the latter it did not attain statistical significance, possibly due to the smaller number of cows involved (n=5 in Experiment 2 versus n=15 in Experiment 1). Experiment 2 therefore needs to be repeated with a higher number of cows per group.

In Experiment 1 (early-mid lactation), the differences observed in milk and MS yield were due to a white clover main effect and to a ryegrass cultivar x white clover interaction (Table 2.7). Treatments containing white clover (HSG+cl and control+cl) produced 10% more MS (1.61 versus 1.45 kg/cow) and 7.5% more milk (20.9 kg/cow versus 19.3 kg/cow) than treatments sown without the legume (HSG and control). Data from Harris et al., (1997) showed that feeding cows with diets containing 50% and 80% of white clover increased milk production by 18 % compared with cows fed 20% of clover, which is the typical situation on NZ dairy farms. In Experiment 1 however, responses to inclusion of clover were much lower than that observed by Harris et al., (1997) as the content of clover in the sward was also very low (Table 2.4). Although treatments with white clover in the present study were sown to represent the typical situation on NZ dairy farms (at least 20% of white clover) the actual clover content was much lower than expected (approximately 8% in Experiment 1 and 2% in Experiment 2). The poor establishment of the clover in the sward was attributed to a long period with wet weather conditions prior to starting Experiment 1.

The inclusion of white clover in the diets also had significant effects on plant chemical composition, mainly decreasing the concentration of NDF and/or ADF and thus increasing the quality of the herbage ingested (ME and OMD). This was further enhanced when combined with HSG. Furthermore, it was shown that the presence of white clover increased N intake in cows

grazing treatments containing the legume (Experiment 1; Table 2.9). This reduction of NDF and increased OMD may increase cow's DMI (Miller et al., 2001) and consequently increase N intake which in turn would possibly offset the potential benefits of HSG, as the amount of N excreted in urine would probably be higher. As the content of white clover in the sward was not the desired proportion, the interaction between HSG and white clover and its effects on milk yield and N partitioning needs to be further investigated in future studies in NZ; better productive responses to HSG may be obtained in the presence of white clover under NZ conditions.

Estimated N intakes were higher in autumn (Experiment 2) than in spring (Experiment 1), consistent with the higher content of CP in autumn herbage. Consequently, the proportion of N intake excreted in urine was higher in autumn (0.56) than in spring (0.31), as was also found by Pacheco et al., (2009) when feeding cows with HSG (cv. AberDart) and a control grass in spring and autumn in NZ. No differences were found in MUN concentration among treatments in both experiments. Milk urea nitrogen concentration has been shown to have a positive relationship with the amount of N excreted in urine by cows (Jonker, et al., 2002). The higher values in Experiment 2 than in Experiment 1 in the present study confirm that the amount of N excreted in urine was likely to be higher in the cows grazing autumn pasture.

One of the most consistent findings in UK studies was a reduction in urinary N excretion (as a % of N intake) in dairy cows fed HSG (Chapter 1; Miller et al., 2000; 2001; Moorby et al., 2006). In contrast to these data but in good agreement to other NZ data (Pacheco et al., 2009) in the present study there was no reduction of N excreted in urine as a proportion of N intake from feeding HSG in early (Experiment 1) or late lactation (Experiment 2).

Those studies which have shown significant improvements in reducing N excretion in urine have also reported significant differences in WSC concentration between HSG and control grasses, always being higher for the HSG compared with a control grass. However, two of these studies have 'artificially' created the WSC differences; through harvesting the control grass in the

morning and the HSG in the afternoon, (when grasses contained 161 g WSC/kg DM and 243 g WSC/kg DM, respectively; Moorby et al., 2006) or by applying N fertiliser to the control grass (194 g WSC/kg DM and 234 g WSC/kg DM for control and HSG, respectively; Miller et al., 2001). Additionally, the CP content in the herbage for all three UK studies ranged from 92 to 145 g/kg DM. In contrast, in the present NZ study the levels of CP were higher and averaged 239 g/kg DM for autumn and 162 g/kg DM for spring. Similar levels of CP were reported by Pacheco et al., (2009) who also did not find reductions in N excretion when cows were fed HSG. This suggests that positive responses in reducing N excretion in urine might be obtained when the diet is higher in WSC but also lower in CP concentration. Miller et al., (2001) stated that when the concentration of CP in the diet is low (as it occurred in the UK studies), animals may benefit more from elevated WSC concentration in the diet as this would improve N utilisation in the rumen, increasing the amount of AA absorbed in the small intestine (Kingston-Smith & Theodorou, 2000) and thus improving animal performance.

From Figure 2.2 A it can be observed that there is a breakpoint in the relationship between herbage CP:WSC ratio and N utilisation in dairy cows, below which the NUEm increases, which was 1.32 in the present study. This was also confirmed by Pacheco et al., (2007) and Edwards et al., (2007) who found similar breakpoint CP:WSC ratios (Figure 1.6 Chapter 1). At this breakpoint, NUEm was similar to that found by Pacheco et al., (2007) (14.2 g milk N/100 g N intake versus 19.8 g milk N/100g N intake, obtained in the present study). Autumn herbage showed the highest CP:WSC ratio and it greatly exceeded that calculated for spring herbage (2.27 versus 0.72). Very high CP:WSC ratios in perennial ryegrass pastures grown in NZ in autumn (above 1.32) may lead to excessive absorption of ammonia from the rumen, reduced rates of rumen microbial protein synthesis (limited by availability of WSC) (Kingston-Smith & Theodorou, 2000) and high ME costs involved in urea excretion (Waghorn & Wolff 1984). It seems that the CP:WSC ratio in herbage has to fall below 1.32 before ammonia production and urea excretion are reduced and

microbial protein synthesis is increased in order for milk protein production to be increased. Further decreases in the CP:WSC ratio below 1.32 were associated with a linear increase in the conversion of pasture N to milk N (Figure 2.2 A), indicating that for high N use efficiency, grazed pasture herbage should contain more WSC than CP, which was the case in the present study for spring pasture but not for autumn pasture. The problem of low N use efficiency is therefore much more marked with autumn pasture than with spring pasture.

To reduce the CP:WSC ratio in a diet and achieve high NUEm, more WSC is required in high CP herbage than in low CP herbage. In our study, to achieve a CP:WSC ratio of 1.32 in spring, when the average CP content in the herbage was 162 g/kg DM, a minimum of 123 g/kg DM of WSC would have been necessary. However, the WSC concentration for Experiment 1 was higher and averaged 226 g/kg DM, giving a CP:WSC ratio of 0.72 which led to a higher NUEm. On the other hand, with high protein autumn pasture (239 g CP/kg DM), to achieve a ratio of 0.72 it is necessary to raise WSC concentration to 332 g/kg DM, whereas the measured average WSC concentration for autumn herbage was only 105 g/kg DM. In the same way, following equation 12 , with a CP:WSC ratio of 0.72 (Experiment 1-spring), NUEu was only 31 g N /100 g N intake, while with a ratio of 2.27 (average for Experiment 2-autumn) cows excreted 55 g N in urine for every 100 g N they consumed.

The objective of increasing WSC up to 332 g /kg DM in order to attain a low CP:WSC ratio in autumn herbage is not feasible using traditional breeding techniques. Results of many years of traditional breeding programs in the UK indicated that breeding perennial ryegrass from 1991 up to 2004, increased mean WSC by 37 g/kg DM (Wilkins & Lovatt, 2004), which represents an annual gain of 3 g WSC/kg DM. However, the increase of WSC concentrations in herbage is usually offset by a decrease in CP concentration (Vibart, 2009; Cosgrove et al., 2009). Cosgrove et al., (2009) calculated that an increase of one unit of WSC concentration in herbage would decrease CP concentration by 0.62 and 0.44 in spring and autumn, respectively. In this way, for

Experiment 2, the CP:WSC ratio of 0.72 for autumn herbage would be achieved with a lower concentration of WSC (approximately 245 g WSC/kg DM) than that proposed earlier.

Therefore, decreasing the CP:WSC ratio of NZ pasture can be done by increasing the levels of WSC but at the same time decreasing levels of CP. To achieve a CP:WSC ratio that is below the breakpoint postulated in the present study, one possibility would be reducing CP content in autumn herbage by 50 g/kg DM and increasing WSC content up to 100 g/kg DM. In this way, for the concentrations of CP and WSC attained in Experiment 2 (autumn) the new CP:WSC ratio would be close to 0.92 and this would allow for a higher NUEm. This should be considered as the objective of producing a NZ HSG.

Producing a HSG in NZ with these characteristics for autumn pasture is likely to be very costly, in terms of both money and time. One way of checking the validity of these calculations would be through giving graded levels of ruminally infused sucrose to stall fed lactating dairy cows fed autumn pasture and studying N secretion in both urine and milk. This should define the CP:WSC ratio that gives the best conversion of dietary N to milk N and also reduces urinary N excretion.

Our experiment compared the response of cows in terms of milk yield and N partition when fed two different types of grass sown with or without white clover. It is important to highlight that the HSG cultivar used in these experiments has been imported from the UK and it has obviously not been selected for NZ conditions. Thus, in the present study a UK-derived HSG was tested against a NZ-derived control ryegrass; so the two cultivars may have differed in other agronomic characteristics as well as in WSC content. The expression of the 'high sugar' trait could not be confirmed in this study, as our results showed that the levels of WSC were not always higher in the HSG than in the control.

An analysis that compared NZ and UK climatic conditions showed that the latter has lower annual average temperature than NZ (Parsons et al., 2004). A study conducted in NZ investigated the expression of the 'high sugar' trait outdoors and indoors with the HSG cultivar (AberDart) and a

control (Parsons et al., 2004) and their results suggested that low temperatures may be necessary for expressing higher levels of WSC in ryegrass in NZ. This clearly indicates that it is necessary to identify or select cultivars that can express the 'high sugar' trait under NZ climatic conditions. Not only higher levels of WSC are desirable but also the selection of a 'high sugar grass' in the future must be orientated to develop a cultivar with higher levels of sugars and with lower levels of CP, thus a lower CP: WSC ratio.

2.5 Conclusions

The NZ dairy, beef, sheep and deer industries heavily rely on grazed perennial ryegrass as the main source of feed for ruminant production. Developing a NZ HSG, able to improve N utilisation efficiency in the rumen, would have benefits for both milk and meat production. There is also a possibility of reducing urinary N excretion and hence reducing N losses in the form of nitrous oxide and as groundwater nitrate run off. In summary, a HSG developed for NZ conditions has the potential for improving milk and meat production while reducing the environmental impact of farming ruminant animals under grazing conditions.

Whilst the benefits of HSG in the present study have been small, continuation of this work to produce a NZ selected HSG with a smaller CP:WSC ratio is likely to have major benefits for pastoral farming in NZ. In order to be effective, a NZ derived HSG should increase autumn WSC levels by approximately 100 g/kg DM, whilst reducing CP content by at least 50 g/kg DM. Such a HSG is also likely to have benefits when grazed in spring.

The low content of structural fibre in the treatments containing white clover and especially when combined with HSG were associated with increased total milk and MS yields in Experiment 1, suggesting that under NZ grazing conditions best responses to HSG may be obtained in a mixture with white clover.

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