NITROGEN ISOTOPIC FRACTIONATION AND NITROGEN USE EFFICIENCY IN BEEF AND DAIRY CATTLE

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ABSTRACT of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

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by N.M.Wheadon

Future rises in world population, increased demand for food production and greater concern for environmental emissions means that new strategies are required for sustainable growth of ruminant industries. Improvement of feed efficiency in cattle is a major solution to increasing production at lower costs; however, identifying between-animal variation requires markers to predict their phenotype. The studies reported in this thesis investigated a new approach to predict feed efficiency based on measuring the differential fractionation of the stable isotopes of N (^{14}N and ^{15}N) in plasma and milk. The main objectives were to evaluate the advantages of using nitrogen-use efficiency (NUE; g milk N/g feed N) as a measurement of feed efficiency in dairy cows, investigate the relationship between plasma and milk N isotopic fractionation ($\Delta^{15}N$) and feed efficiency in beef and dairy cattle, and lastly to understand the genetic factors involved in these relationships. Studies were carried out in Ireland and New Zealand, using growing beef heifers and lactating dairy cows in a number of herds, diets based on grass silage or grazed grass, as well as a range of diet composition and production levels.

Chapter 3 showed a highly correlated relationship between NUE and an energy based measure of efficiency (ECE) (r^2 = 0.90; P<0.001), but NUE was less affected by the short term changes in body reserves over lactation so was a more reliable and stable measurement of feed efficiency in dairy cows to investigate the relationship between NUE and N isotope fractionation. Plasma $\Delta^{15}N$ was related to both Feed conversion efficiency (negative) for the whole population (r^2 = 0.35; P<0.001), and repeatable for the subset of animals over four time points in beef heifers (r^2 = 0.47, 0.56, 0.64. 0.56 respectively; all P<0.001) (chapter 4). Plasma $\delta^{15}N$ measurements from the

same animals in the subset were significantly correlated over adjacent time points (P<0.001 correlation between all days) (average r = 0.96).

Further development of the use of N fractionation to predict NUE was extended to free grazing dairy cattle in chapter 5-7. There was pasture and N isotope variation in the 9 treatment groups in chapter 5, with no relationship between NUE and plasma $\delta^{15}N$ or $\Delta^{15}N$ after taking account of this effect. Milk Milk $\delta^{15}N$ (‰) was measured in chapter 6, and the high N isotope signature 7.28 (SD = 0.50) and 7.06 (SD = 0.44) for periods 1 and 2 resulted in a low enrichment of milk $\Delta^{15}N$ (mean 0.64; SD = 0.44) in period 2. There were weak negative correlations between NUE, milk $\delta^{15}N$ and $\Delta^{15}N$ because NUE was heavily driven by a large excess of rumen degradable protein. Chapter 7 demonstrated a highly significant relationship between NUE and plasma $\delta^{15}N$ (r^2=0.23; P<0.01) and $\Delta^{15}N$ plasma (r^2=0.45; P<0.001). There were no significant relationships between NUE and any urine analytes within groups apart from a negative relationship with uric acid (mmol/l) (P<0.05).

There were differences in feed efficiency and $\Delta^{15}N$ results between beef and dairy cattle which were attributed to differences in the efficiency of utilisation of amino acids for growth and lactation respectively, absolute maintenance requirements and the dilution of maintenance for production. There were high levels of Rumen Degradable Protein (RDP) in pasture in most studies which was responsible for the weak relationship between N isotopic fractionation and feed efficiency in some studies (Chapter 5 and 6). Results suggested that $\Delta^{15}N$ may be an indicator of the genetic variation in animal efficiency of amino acid utilisation in body tissues, but it was not strongly related to NUE because of the dilution effects of excess RDP. Preliminary evidence also suggested that differences in N isotope fractionation are a result of genetic between-animal variations in feed utilisation (Chapter 6 and 7). However, further investigation is required with more complex models to evaluate sire differences and relationships between parents and progeny. High Breeding Worth (BW) was associated with more N efficient animals at lower intake levels. Selection for cows based on BW may indirectly increase feed efficiency; in particular NUE, because protein yield is an important trait in the BW index and has a

high economic weighting, however this process may still be slow because of genetic correlations with other traits in the index.

The main conclusions of this thesis were the reliability of using NUE as a measurement of feed efficiency in dairy cows, and the highly significant repeatable negative relationships between plasma $\Delta^{15}N$ and feed efficiency (measured as FCE; g live weight gain/ g intake) in growing beef heifers (Chapter 4) and in lactating cows (measured as NUE) (Chapter 7). $\Delta^{15}N$ in plasma has potential to be a used as a diagnostic tool in breeding programmes, evaluating feed efficiency without measuring feed intake (and diet composition). However, this approach may be limited by the ability of using isotope fractionation to detect variation for diets rich in RDP and requires further study with diets containing lower levels of nitrogen.

Keywords: cow, heifer, stable isotope, nitrogen use efficiency, feed conversion efficiency, energy conversion efficiency, nitrogen metabolism, rumen degradable protein, metabolisable protein, urea nitrogen, milk protein, sire, breeding index, body reserves, dilution of maintenance, phenotype

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Ph*inally* **D***one*.
Go n-éirí an bóthar leat
Whakawhetai, me te waimarie pai

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

COMMON ABBREVIATIONS USED

Acid detergent insoluble nitrogen fraction	ADIN
Blood urea nitrogen	BUN
Breeding worth/ Breeding value	BW/BV
Crude protein	CP
Delta per mil (molecules per thousand)	δ or ‰
Enrichment (for nitrogen: $\Delta^{15}N$ = animal tissue $\delta^{15}N$ ─ diet $\delta^{15}N$)	Δ
Digestible organic matter in total dry matter	DOMD
Dry matter	DM
Dry matter intake	DMI
Dry matter digestibility	DMD
Energy conversion efficiency	ECE
Feed conversion efficiency	FCE
Feed conversion ratio	FCR
Lactation worth/ Lactation value	LW/LV
Live weight	LWT
Metabolisable energy	ME
Metabolisable protein	MP
Metabolisable protein efficiency	MPE
Milk urea nitrogen	MUN
Nitrogen	N
Nitrogen isotopic fractionation	$\delta^{15}N$
Nitrogen use efficiency	NUE
Organic matter digestibility	OMD
Production worth/ Production value	PW/PV
Quickly degraded protein	QDP
Residual feed intake	RFI
Rumen degradable protein/nitrogen	RDP/RDN
Slowly degraded protein	SDP
Stocking rate	SR
Undegraded dietary protein/nitrogen	UDP/UDN

CHAPTER 1

INTRODUCTION

The dairy sector is the largest contributor to environmental emissions in the agriculture industry (Bleken et al., 2005; Van Passel, 2007). It is difficult to be both environmentally and economically sustainable when there is an increasing demand for food production to support the rising population (Clark and Davis, 1983), and when animal feed represents over half of farm production costs (Linn et al., 2004). Global awareness of greenhouse gases (Nevens et al., 2006) and new government legislation (Powell, 2009) means that new strategies and solutions are required to continue to be sustainable. Low cattle feed efficiency, defined as units of output per unit of input, is a result of the inefficient use of dietary nutrients, in particular nitrogen (N) where up to 90% can be excreted as waste in urine and faeces, affecting both ground water and air quality (Chase, 1994; Castillo et al., 2000). Nitrogen-use efficiency (NUE; g milk N/g N intake) is low on dairy farms in both New Zealand and Ireland (Watson and Atkinson, 1999) which is predominantly caused by the high N content of pasture in grazing systems, relative to animal requirements for production.

Milk and meat protein are the most valuable components to food production so a major solution is the improvement of feed and N efficiency in livestock. Limited progress to-date is a result of the complex nature of feed efficiency traits that are influenced by a number of environmental and animal (genetic) factors, combined with the high costs of collecting large amounts of animal data. Therefore, identifying between-animal variation in feed efficiency requires simple and cost effective diagnostic tools for use with large groups of animals. There have been recent successful insights into genetic markers for feed efficiency (particularly RFI) in New Zealand (Pryce at al., 2012) but previous work has not found reliable metabolic predictors of feed efficiency or components of, in particular for dairy cows. These phenotypic markers would be extremely valuable in identifying animals that are

divergent for increased nutrient utilisation and predicting efficiency in animals where intake or diet composition is not known.

A new approach, based on measuring the differential fractionation of the stable isotopes of N (^{14}N and ^{15}N) can indicate the partitioning of N in metabolic pathways leading to milk or meat protein and urinary N respectively (Steele and Daniel, 1978). Nitrogen fractionation is expressed as the ratio change from a heavy to light isotope using delta (δ) units (molecules per thousand) or using ‰. When the fractionation ratio is greater than 1, the tissue is considered enriched, and the enrichment factor symbol Δ is used. In the case of enrichment of $\delta^{15}N$, $\Delta^{15}N$ = animal tissue $\delta^{15}N$ ─ diet $\delta^{15}N$.

Initial work investigating the dietary factors affecting N fractionation in lactating sheep, goats and dairy cows (Cheng, 2012) forms the basis of this work. In this thesis, further studies were developed to relate isotope measurements to NUE in lactating cows and beef cattle with the ultimate goal of developing a sampling protocol for free-grazing animals. The underlying genetic effects of feed efficiency and N isotopic fractionation were assessed using breed and ancestry records to establish the suitability of this measurement in selection, and its heritability.

The first chapter of the thesis (Chapter 2) is a review of the literature which gives an understanding of dietary protein sources, N metabolism, N fractionation, existing markers, and knowledge of the genetics of feed efficiency. Gaps in the literature are identified and the objectives of the thesis are made. In Chapter 3, two methods of measuring feed efficiency in dairy cows are evaluated. In Chapter 4, N isotope fractionation is investigated for its potential to predict feed efficiency in growing beef heifers. Chapters 5 and 6 investigate the same approach using field studies conducted in Ireland and New Zealand and on free grazing dairy cows. In addition in chapter 6, sire group differences are studied. In chapter 7, a further investigation is conducted into the relationship between NUE and N isotopic fractionation in a recorded intake trial between mother and daughter pairs to assess heritability of

NUE. The general discussion summarises the main findings from the thesis chapters, including conclusions and future recommendations.

The objectives of this thesis were:

1. Evaluate the reliability of using NUE as a measurement of feed efficiency in dairy cows by comparing NUE with an energy based measured of efficiency (energy conversion efficiency; ECE) over an entire lactation cycle and to understand the effect of diet and genetics on N and energy metabolism.
2. Investigate the relationship between N isotopic fractionation and feed conversion efficiency (FCE; g LWT gain/ kg DM intake) of growing beef heifers and its potential to provide a rapid low-cost estimate of FCE in large groups of cattle.
3. Investigate the relationship between N fractionation and NUE in free grazing lactating dairy cows and assess the effect of breeding worth and sire on NUE and metabolisable protein efficiency (MPE) and their relationship with N isotope fractionation.
4. Investigate the relationship between N isotopic fractionation and NUE in parent and progeny pairs of dairy cattle.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

A major potential solution to increasing food production in a resource constrained environment, whilst being enviromentally and economically sustainable, is the improvement of feed and nitrogen (N) use efficiency in livestock. There is a demand for simple and cost effective diagnostic tools to measure feed efficiency in ruminants, which to be useful in breeding, also needs to be heritable and repeatable in progeny. It is important to understand the physiological basis of metabolic markers to understand the nutritional, environmental and genetic factors that contribute to nutrient use in cattle. In this review, the contribution of N to global emissions of greenhouse gases is summarised, along with the reasons for interest in measuring the utilisation of nutrients in cattle. Methods of measuring feed efficiency are described and the sources of variation between individuals, including the underlying metabolism and genes that contribute to this trait. The complex nature of N cycling in the ruminant makes it is difficult to find phenotypic markers that can detect variation in N partitioning, in particular for free grazing animals as intake and diet composition cannot be controlled. As a result of these difficulties, indicators of feed efficiency are weak. There are gaps in the literature, in particular in the area of genetics of feed efficiency in ruminants. In particular, there is much less work on the genetics of feed efficiency in ruminants. A new approach, N isotopic fractionation, which is related to the underlying N metabolism and cycling, is introduced as an alternative method for predicting feed and N efficiency in cattle.

2.2 Environmental impact of nitrogen

Ruminants that consume high amounts of dietary N that is not efficiently converted to milk or meat protein result in a large excess of N excreted in the faeces and urine (Powell et al., 2010; Cheng et al., 2011), ultimately releasing N to the environment

(Chandler, 1996). Nitrogen affects the air (as ammonia and oxides), surface, and ground water (from leaching nitrates) (Tamminga, 1992). Nitrogen utilisation is extremely variable and can range from 5% to 45% of feed N converted to animal protein. The remaining portion is excreted as waste and a proportion of this is recycled to provide a source of nutrients to plants (Oenema and Tamminga, 2005). The estimated global waste of N voided by ruminants is between 80 and 130 teragram (10^{12} g) per year and 60% of this is caused by cattle farming (Bleken et al., 2005). The agriculture industry also contributes heavily to ground and surface water pollution and increases nutrient surpluses (Ramirez and Reheul, 2009). Approximately 40 to 50% of N excreted in manure is in the form of urea and ammonia N when excreted in urine (Van Horn, 1994). This can cause excessive accumulations of ammonia in the urine, of which 50 to 75% can be lost from manure before nitrification (Van Horn, 1994). Storage and management of waste varies between farms (Powell, 2010), which makes efforts to define best practice difficult (NRC, 2001). These techniques are based on animal movement (indoors/outdoors), animal type (lactating, dry) and seasonal variations (Powell, 2009).

2.3 Measuring the utilisation of nutrients

There is interest in measuring the utilisation of nutrients in cattle because there are environmental and economic consequences of inefficiency. In order to assess the contribution of cattle to N emissions, measuring utilisation of feed N to product N is a useful method to indicate the wastage incurred. The route of waste (mainly urine and faeces) is also important from an environmental point of view as urine is more volatile and mobile than faeces. There are several methods to measure feed efficiency, defined as the amount of feed consumed per unit of output (milk, meat). It is a complex trait that has often been over simplified when measured in ruminants (Archer et al., 1999) by overlooking many factors that contribute to this trait e.g. environment, maturity and health (Arthur et al., 2004). A description of the 3 most commonly used feed efficiency measures are outlined below and their suitability for beef and dairy cattle is discussed.

2.3.1 Feed conversion efficiency

Feed conversion efficiency (FCE) is defined as a unit of gain per unit of intake. In the case of dairy cattle, gain is considered as kg of milk solids whilst in growing beef cattle (and other cattle) is measured as body mass gain. Feed conversion ratio (FCR) is defined as the ratio of feed intake to live weight (LWT) gain or feed input per unit output (Crews jr, 2005) and is a traditional method of measuring feed efficiency used mainly for growing animals. Feed intake and FCE are genetically and phenotypically correlated with animal growth traits (Crews jr et al., 2006) and the genetic correlation between FCE and LWT gain ranges from -0.24 to -0.95 (Koots et al., 1994), so selection for FCE increases growth rate and mature size (Crews jr, 2005). It is assumed that animals selected for increased growth will have higher FCE, and whilst this appears beneficial in such instances as beef cattle, selection for this trait does not automatically lead to an increase in feed efficiency. Archer et al. (1999) demonstrated that there may be antagonistic correlated responses between feed to gain ratio and other traits, and additionally, Gunsett (1984) found that the complex factors involved in this trait means there may be an uneven distribution of selection between these other correlated traits leading to unpredictable responses to genetic selection.

2.3.2 Residual feed intake

Residual feed intake (RFI), defined as the difference between an animal's measured intake and expected intake based on its weight and growth rate, has been considered a more robust measurement of feed efficiency in beef cattle as it uses recorded feed intakes and adjusts for maintenance requirements and body weight (Koch et al., 1963). Residual feed intake measures the partitioning of feed to production, where the residual portion is the feed consumed that is not accounted for or explained by growth or body weight (Crews jr et al., 2006). Residual feed intake measurements are phenotypically independent of the traits used to calculate expected feed intake, which allows a comparison to be made between individuals in a group (Herd and Arthur, 2009). Animals with higher efficiency have lower feed intakes than expected

based on their growth rate and body weight (RFI less than 0) (Crews jr et al., 2006). Residual feed intake has been useful to identify beef cattle with lower feed intake based on the same production levels (Kelly et al., 2010), however there are high costs and technical aspects associated with measuring this trait (Moore et al., 2009). Measuring intake for both FCE and RFI involves a recommended duration of 35 days, with at least a 70 day test for recording growth rate (Archer et al., 1997).

Both FCE and RFI are less suited to measuring feed efficiency in dairy cows because they are correlated to LWT and there are changes in body reserves over the lactation cycle (Buckley et al., 2003). The contribution of body reserves can be up to 30% of milk constituents so has a large effect on FCE over a short period of time. FCE is measured as a unit of live weight gain per unit of intake, where muscle protein is the most profitable, whereas in dairy cattle the interest is in milk solids per unit of intake and not the live weight of the cow.

2.3.3 Nitrogen-use efficiency

Nitrogen use efficiency (NUE) is defined as the conversion of dietary N into product N (milk or meat) and is commonly in the region of 20% for European cattle (Powell et al., 2010) but is often lower than this in New Zealand systems. It can be used in beef cattle (although it is difficult to measure muscle protein N) and is a more useful measurement in dairy cows because it relates to milk protein output which is the most valuable component of milk. NUE can also be measured on a whole farm basis (total input N/total output N) and can range from 8% to 64%.

Improving NUE in dairy cows will reduce urine N excretion and as a result reduce leaching into water tables and emission reservoirs (Galloway, 2003). NUE is a simple and non-invasive method of measuring nutrient utilisation in dairy cattle (Cheng et al., 2011). Increasing NUE can be achieved by manipulating dietary N and increasing the genetic potential of the herd. In order to utilise the animal variation in feed efficiency complete knowledge of the underlying physiological causes are not

always necessary (Arthur et al., 2004), but it is still important to understand genetic relationships at different stages of the life cycle and through generations.

2.4 Factors that affect feed efficiency

To understand the underlying metabolic basis of feed efficiency in beef and dairy cattle, it is important to understand the sources of between-animal variation. There are many factors that contribute to this variation including dietary intake (including quality and quantity), digestibility, metabolism of nutrients, physical activity and thermoregulation (Herd and Arthur, 2009), and the additional changes in physiological state for dairy cows across the lactation cycle. The main contributors to variation in feed efficiency are summarised below. The headings and sub-heading from section 2.4 to 2.7 discuss feed efficiency in different terms, consisting of some sections including only NUE, some only feed efficiency or some sections as a mixture of both to varying degrees.

2.4.1 Dietary protein supply

In ruminants, dietary protein has three main routes of metabolism which are (i) microbial fermentation in the rumen, (ii) absorption in the small intestine and (iii) catabolism and excretion of excess digestible protein into faeces and urine N (ARC, 1980). Metabolisable protein is used efficiently provided that it is close to the requirements of the animal and that there is enough energy available in the diet. An excess of metabolisable protein relative to energy can be as detrimental to efficiency as an excess of RDP in the rumen. Microbial fermentation allows the dairy cow to utilise low amounts of dietary protein for milk production (Broderick, 2009) and to use non-protein nitrogen (NPN) to synthesise microbial protein (Moran, 2005). The amount of microbial protein synthesised in the rumen depends on the supply of rumen degradable N (RDP) and fermentable energy in the rumen (Figure 2.1). Some protein will not be degraded in the rumen (RUP) and will pass to be digested in the small intestine. Protein degradation in the rumen involves hydrolysis of peptide bonds by endo and exo peptidases and deamination of these amino acids by microbes

(Haque et al., 2012). Digestion of protein in the small intestine is generally high, though N associated with dietary fibre (ADIN) is often less digestible. It is important to formulate diets using digestibility and degradability analysis to reduce inefficient utilisation of N during fermentation and maximise rumen N recycling (Kirchgessner et al., 1994). Both RDP and CP supply will be high from New Zealand pastures, so are both responsible for causing an excess of N that is converted from ammonia to urea to be excreted in the urine. The dominant factor determining NUE is N intake, which is likely to be highly correlated to RDP supply, so NUE is related to both factors.

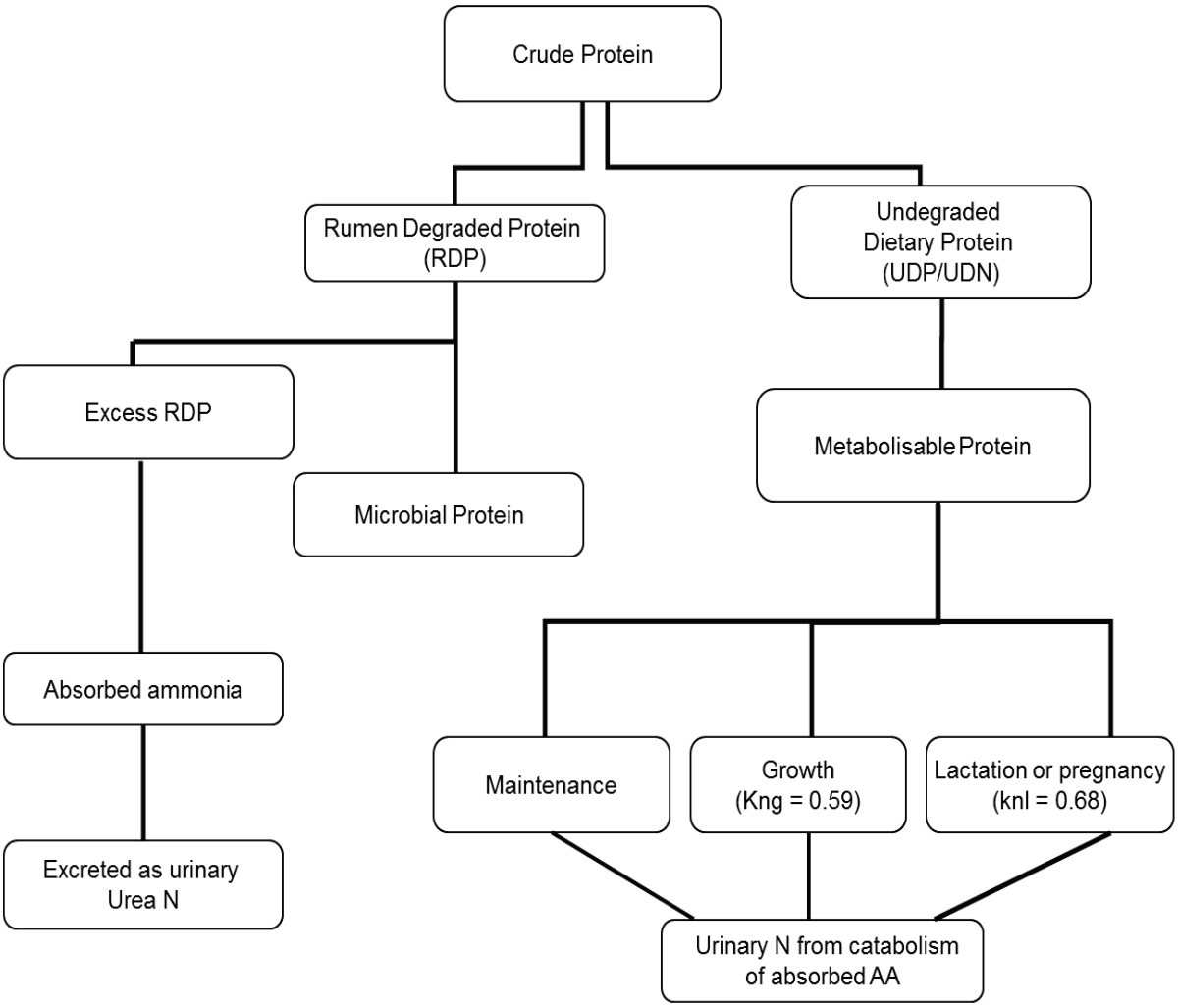


Figure 2.1 Diagram of the supply of dietary protein in the ruminant (adapted from ARC, 1992).

It is difficult to control the intake of protein in grazing animals because pasture often contains more protein than recommended levels (16%) (NRC, 2001); this reflects the use of N fertiliser for grass growth and the presence of legumes with high N content in pastures. There is a diminishing returns relationship with the increase in milk N output in response to additional dietary N, declining as N intake increases (Dibb, 2000; Roberts, 2008). Increasing feed intake to increase milk production leads to a moderate increase in milk N, but a linear increase in urinary N excretion (Bockmann et al., 1997, Kebreab et al., 2001). Low levels of dietary crude protein (CP) can improve NUE and also reduce feed costs (Chase, 1994), but an adequate level of N is still required for carbohydrate digestion in order to make use of the energy supply in the feed (Oldham, 1984). In addition, the quality of feed N consumed will affect utilisation (Huhtanen and Hristov, 2009). Studies with male Wistar rats found increased utilisation of amino acids when fed a high protein diet but the level of utilisation was dependant on intake and type of dietary protein (Sick et al., 1997). Cabrita et al. (2011) found feeding diets with 16% CP increased intake and decreased NUE compared to diets with 14% CP in lactating dairy cows.

2.4.2 Physiological factors that contribute to feed efficiency

A large contribution to variation in feed efficiency has been attributed to locomotion. Heat maintenance and locomotion explained up to 73% of variation in efficiency in Angus steers selected for RFI (Herd and Arthur, 2009) and up to 80% of the variation in chickens (Luiting et al., 1991). Pedometer correlations with RFI in cattle showed that up to 10% of the variation in RFI was dependant on physical activity (Richardson, 1999). Animals with higher efficiency have also been found to have up to a 22% reduction in feeding events (Kelly et al., 2010).

Dairy cows that have been selected for high milk yield generally have faster digestion and absorption of dietary nutrients (Adams and Belyea, 1987) and there is variation in the production of microbial protein, resulting in differences in the supply of amino acids (Khan et al., 2000). Selection for young bulls and heifers on the basis

of RFI resulted in differences in estimated dry-matter digestibility (DMD), used to indicate feed utilisation (Richardson et al., 1996). Richardson et al. (2001) found that progeny of steers that were selected for high RFI also had higher whole body chemical fat and protein than animals with lower RFI.

2.4.3 Contribution of body reserves to feed efficiency

Feed efficiency in dairy cows is particularly difficult to interpret because of the mobilization and replenishment of body reserves that occurs during lactation and late gestation (Madhav et al., 1997; Friggens et al., 2004; Prendiville et al., 2009), especially during early lactation when utilisation of body reserves is highest (Dewhurst et al., 2002). Higher efficiency in early lactation is the combined effect of the utilisation of body reserves (which can be responsible for approximately 30% of milk production during this time; Bauman and Currie, 1980), and the spreading of maintenance costs across more milk production (Vandehaar and St-Pierre, 2006). As feed intake and production level increase, the proportion of feed nutrients used for maintenance purposes becomes smaller, where intake reaches a maximum point of efficiency and follows the laws of diminishing returns. There are relative differences in maintenance costs for energy and protein (ARC, 1980; AFRC, 1992).

The quantity of body protein that is mobilized and replenished appears to be much less than body fat, though the pattern of change across the lactation cycle appears similar (Andrew et al., 1994; Moorby et al., 2002), and there is considerable variability in the conversion of nutrients to milk, even within groups of animals offered the same diet (Davey et al., 1983). Schröder and Staufenbiel (2006) estimated that for each unit of change, fat and protein contribute 93 and 7% respectively to total tissue loss or gain and 1kg of tissue represents 0.64kg fat, 0.28kg water and only 0.08kg of protein (Madhav et al., 1996). Immediately post partum, it has been estimated that dairy cows can utilise up to 1000g/d of protein to sustain the mammary gland (Bell et al., 2000) and N balance measurements have reported up to 21kg of protein is mobilised in early lactation (18% CP diet) (Botts et al., 1979). In contrast, mobilization of body fat is much greater than protein during early lactation

(50-60kg; Smith and McNamara, 1990). These differences reflect the genetic variation in the regulation of muscle mass (Lee and McPherron, 1999; Bell et al., 2000) and body fat (Cases et al., 1998; Schennink et al., 2007; Prokesch et al,. 2009; Thering et al., 2009) over different periods of lactation.

Increasing available energy in concentrates does not lead to a reduction in the loss of body reserves during early lactation (Friggens et al., 2004). Williams et al. (2013) found greater tissue energy retention and tissue energy (MJ/kg of $LWT^{0.75}$) as a percentage of gross energy intake when adding grain to the diet of dairy cows, and although there was an increase in milk energy, efficiency of use of the supplement for milk production was unchanged. An increase in retained energy with days in milk is expected as cows replace energy stores mobilized in early lactation (Williams et al., 2013).

Body reserves and animal behaviour are factors that are difficult to account for when measuring feed efficiency. Dietary protein can be manipulated in animals that are fed on mixed rations, but not in free grazing dairy cows. There has been a lot of research on the contribution of the diet to feed efficiency (Danes et al., 2013), and specifically N efficiency (e.g. Castillo et al., 2000; Kebreab et al., 2001; Drackley et al., 2006), but less work on genetics of feed efficiency, especially in N efficiency which has also mainly been in other species such as pigs and poultry (Arthur and Herd, 2005). This is because of the difficulty in collecting feed efficiency data in large groups of animals because of the high costs associated with measuring feed intakes. Most feed efficiency measurements are constrained to a selected period of growth or lactation which makes it difficult to assess long term efficiency.

2.5 Understanding efficiency at a genetic level

Overall efficiency needs to be understood on a systems biology level that incorporates genetic, nutritional and environmental interactions (Rocco and McNamara, 2013). It is important to consider each subset system which plays a role in the overall metabolic state of the animal and its production level response (McNamara, 2012). However, it is difficult to study empirical measurements that are a priority for selection (e.g. muscle gain, milk yield) in combination with molecular measurements that link to these traits in order to understand expression of genes with production levels (Koltes and Spurlock, 2011). There has been ongoing research in New Zealand that has identified differences in efficiency (RFI) between animals both at a production level and a genomic level (Pryce et al., 2012). Improvements in efficiency are possible if we target areas of genetic variation in individuals (Baldwin, 1980) and select animals that have increased capability for nutrient conversion (Danes et al., 2013). This could be achieved by increasing the dilution of maintenance costs and improving efficiency of metabolic systems e.g. liver and adipose tissues (McNamara, 2012). Cows of differing genetic merit have been shown to differ in intake and milk yield (especially when restricted), and there is a positive relationship between utilisation of body reserves and higher milk production in higher merit cows (McNamara, 2012; Khan et al., 2013). Adipose tissue has a large influence on homeostasis (Prokesch et al., 2009) and the variation in energy use for metabolism can be up to 100% between animals (McNamara, 2012) because of differing ability to store and mobilize fat stores (Khan et al., 2013).

Protein and fat anabolism and catabolism need to be considered as a kinetic and dynamic systems, with constant changes in amino acid and adipose cell fluxes and concentrations (Cornish-Bowden, 2005). Release of energy occurs during digestion, absorption, storage, mobilization and the synthesis and hydrolysis of the pyrophosphate bonds of ATP (Baldwin et al., 1978a-c). Overall energy efficiency of milk synthesis is an estimated 83%; the energy expenditure in adipose tissue is 527 KJ per mole of triglyceride turned over and the metabolic efficiency for milk fat synthesis is 72%. ATP expenditure for protein synthesis is approximately 1884

kJ/day (75 KJ per mole x 500g synthesised protein a day) and the metabolic efficiency for milk protein is 82% (Baldwin, 1968).

2.5.1 Genetic control of protein and fat metabolism

During early lactation dairy cows are in a state of negative energy balance because there is a higher demand for milk fat synthesis (Loor et al., 2006; Sumner and McNamara, 2007; Koltes and Spurlock, 2011) and consequently, adipose catabolism is highest at this time. This causes a significant increase in the expression of genes related to nutrient utilisation and lipogenesis and a decrease in expression of genes for anabolic control (Sumner-Thomson et al., 2011; Rocco and McNamara, 2013). Milk production is supported by enzymes controlling lipogenesis and lipolysis in response to changes in demand from the mammary gland (Rocco and McNamara, 2013). The mechanisms controlling lipogenesis, adipogenesis and lipolysis are independent systems, and occur at different speeds and times during lactation (Khan et al., 2013). Lipolysis is positively correlated to milk energy output and negatively correlated to energy intake (McNamara and Hillers, 1989) and is controlled by the activation of protein phosphorylation and gene transcription. In contrast, lipogensis is coordinated with energy intake and decreased activation of gene expression for anabolic enzymes (Rocco and McNamara, 2013).

During early lactation, there are changes in the expression of key genes and hormones to regulate lipogenesis and pathways of anabolism of triacylglycerol (Thering et al., 2009) e.g. acetyl-CoA carboxylase, lipoprotein lipase and insulin (Drackley et al., 2006; McNamara, 2012), fatty acid binding protein-4 (Damcott et al., 2004), ß-3 adrenergic receptor, NEFA, BHBA, myostatin and leptin (Reist et al., 2002). Myostatin has been identified as a key driver in the TGF-ß group of factors involved in growth and differentiation of muscle mass (Lee and McPherron, 1999). Mobilization of protein tissue is under endocrine control by a range of regulators e.g. insulin, IGF-1 and binding proteins. Lipolysis in early lactation is predominantly controlled post-translationaly in response to stimulation of the ß-2 adrenergic receptor (Sumner-Thomson et al., 2011), which provides fatty acids to the mammary

gland. Basal lipolysis increases post partum and there is an increase in expression of ß-adrenergic receptors which change at different stages of lactation (Sumner and McNamara, 2007). Higher genetic merit cows have increased response to ß-adrenergic stimulation and hormone sensitive lipase (McNamara and Hillers, 1989).

Three main proteins control lipolysis; B2AR, HSL and PLIN1 (Rocco and McNamara, 2013), which fluctuate during lactation. Loor et al. (2006) identified 85 genes that were expressed in response to changes in energy intake prepartum. These were genes associated with pathways involving adipose lipid mobilization and increases in NEFA in serum, uptake in the liver, increased oxidation to ketone bodies and CO_2 and increased liver triacylglycerol. Micro RNA expression for lipogenic enzymes, transcription regulators and rates of lipogenesis decrease in early lactation whereas they increase for lipolysis (Khan et al., 2013). There is variation between animals in mRNA transcripts associated with lipolytic control e.g. Ca channel sub unit, ß-2 adrenergic receptor and HSL (McNamara, 2012). Recent work has identified lipolytic proteins in adipose tissues that regulate and coordinate lipolysis at different stages during lactation including hormone sensitive lipase, perilipin, adipose triglyceride lipase and comparative gene identity-58 (Koltes and Sprulock, 2011; Sumner and McNamara, 2011).

2.5.2 Is feed efficiency heritable?

Selection for desired traits targets breeding animals as these are more likely to be passed onto progeny (Wood et al., 2004). Predictors of feed efficiency must be robust and reliable and also need to be heritable, and repeatable in progeny to be used in selection. It is important to obtain estimates of genetic correlations to understand the response of the phenotype to genetic selection (Lande and Price, 1989), and to show which traits should be included in breeding values (Spasic et al., 2012). Unfortunately, feed efficiency (and NUE) are complex traits and selection has been difficult to achieve (Spasic et al., 2012) partially because there is often difficulty in gathering data for heritability analysis (Bormann and Wilson, 2010).

Selection for feed efficiency in cattle has been difficult because of the interaction of multiple genes and because it is not possible to select for a single component trait without affecting the response of other traits (positive or negative) (Crews jr et al., 2006). Data that involves the genetic co-variances of feed efficiency with relevant traits would be beneficial (Archer et al., 1999). In the study by Arthur et al. (2001), genetic selection for low RFI animals resulted in offspring that had a lower intake, were slightly leaner, but in the early growing period had the same feed efficiency as the higher RFI animals (Arthur et al., 2001). Other studies have also found only small effects of selecting for RFI on carcass fat and muscle composition traits (McDonagh et al., 2001; Richardson et al., 2001).

Heritability of RFI and FCE has ranged between 0.16 and 0.58 in growing beef cattle, and heritability of NUE in dairy cows has ranged from 0.07 to 0.40 (Table 2.1). Despite some relatively high estimates, there have been low phenotypic correlations between RFI and production and intake traits (Zamani et al., 2011). The genetic correlation between feed intake and FCE is also low (0.31) (Arthur et al., 2004) because significant genetic variation can occur between individuals (Archer et al., 2002). Feed efficiency is negatively correlated to LWT measures (Parke et al., 1999) suggesting that selection for lower LWT would increase efficiency and reduce maintenance costs and therefore increase profitability.

Table 2.1 Literature estimates of heritability (h^2) and reported s.e. for measures of feed efficiency in beef and dairy cattle.

Reference	Animal description	*n*	Efficiency Measurement	h^2
Herd and Bishop, 2000	Growing beef cattle	540	RFI	0.16±0.08
Arthur et al. 2001		792		0.39±0.04 to 0.43±0.06
Koch et al. 1963		1324		0.28±0.11
Schenkel et al. 2004		2284		0.38±0.07
Crews jr et al. 2003		641		0.58±0.20
Liu et al. 2000	Male and female	282		0.29
Arthur et al. 2001	Male and female	1180		0.39±0.03
Arthur et al. 2002	Mature female	751		0.23
CRC, 2001	Steers and females	2155		0.18
Brelin and Brannang, 1982	Growing beef cattle	235	FCE	0.35±0.24
Mrode et al. 1990		452		0.33±0.10
Fan et al. 1995		534		0.16±0.14
Buttazzoni and Mao, 1989	Lactating cows	79	Net energy efficiency, kg/kg	0.32-0.49
Veerkamp et al. 1995		204		0.30-0.38
Agreeb, 1999		114,351		0.10±0.01 to 0.31±0.03
Zamani et al. 2011		501	Gross efficiency of CP, kg/kg	0.07±0.05
			CP balance, kg/d	0.40±0.071
			Residual protein intake, kg/d	0.03±0.093

Residual feed intake is moderately heritable in cattle (estimated at 0.39) and it has been genetically correlated to feed intake (0.69) (Arthur et al., 2001). There is genetic variation in feed intake e.g. genetic correlations for feed intake and RFI were different when measured in animals that were post-weaning (0.72) and mature (0.98) (Arthur et al., 2004). There are a number of factors involved in the genetic and phenotypic variation in which animals locate, ingest, digest and metabolise feed energy for various processes for growth, maintenance and reproduction (Arthur et al., 2004). There may be similar heritability within a breed group, but differences in genetic variation between breeds, so genetic progress may be faster in groups with lower variation (Vleck and Bradford, 1965).

Theoretically inheritance is most easily assessed by comparing parents and progeny, however there are bias effects that can affect the relationship e.g. maternal effects; offspring phenotype is influenced by parent phenotype which is also affected by external conditions (Muller et al., 2012), and adaptive maternal effects; adaptations to the external environment that are passed on from mother to daughter, which means that past, present and future environmental conditions can effect the phenotype of both mother and daughter (Muller et al., 2012). Heritability estimates suggest the portion of variance that is due to genetics. There has been a lot of research on the environmental sources of variation in maternal effects, however as well as environmental influences, maternal effects also have a genetic component, but there is a lack of research on heritability of maternal traits and the influence of these traits in the phenotype of offspring (Muller et al., 2012) as these are complex studies that require data across a series of generations. It will be important to determine the correlated response to selection based on feed efficiency to see the effects on other traits in a selection index (Case et al., 2012).

2.5.3 Using genome wide association studies for feed efficiency selection

Genomic data has allowed scientists to identify many small gene regions and preliminary genetic markers that are associated with feed efficiency which can be used in gene assisted evaluations (Moore et al., 2006). Recent New Zealand data has found several SNPs on chromosome 14 that may be associated with gene NCOA2, which is involved in energy metabolism (Pryce et al., 2012). The advance of whole genome association studies (GWAS), increase in single-nucleotide polymorphism (SNP) analysis and reduction in cost, have increased the analysis of tiny variations in thousands of alleles that can be investigated for trait associations (Arthur et al., 2004). Single SNP associations with RFI have provided an insight into potential gene regions that may be associated with other measurements of feed efficiency (e.g. NUE).

One of the first studies that investigated differentially expressed genes between animals divergent for RFI identified 181 probes in micro array analysis that were differentially expressed between high and low RFI animals (Chen et al., 2008), among which 85 were up-regulated and 76 down-regulated in the high efficiency group. Seven significant gene networks were linked to RFI with functions including cell growth, protein, fat and carbohydrate pathways, and cancer and drug metabolism. Barendse et al. (2007) also found a high number of regions of the bovine genome associated with RFI in 7 beef breeds. DNA variants linked to energy use were ten times more frequent than those affecting appetite and body mass homeostasis, and it was suggested that there is a trade off between feed efficiency and tissue construction, allowing more energy for other evolutionary traits. There was also a significant difference in the type of micro-RNA region in the SNP sequence which suggests specific regions that are unique to the control of RFI (Barendse et al., 2007). Sherman et al. (2009) mapped quantitative trait loci (QTL) in beef cattle and found 19 chromosomes that contained associations with RFI. Sherman et al. (2010) also identified 150 SNP associated with RFI however only 1 SNP correlated to a previous study by Barendse et al. (2007). Despite this, 9.5% of

the SNP were within 5cM of previous QTL locations found in association with RFI (Sherman et al., 2008).

In contrast, a multifactor study that analysed associations between feed efficiency and feed behaviour, metabolites and body composition found that grouping animals for high and low RFI had no effect on plasma concentrations of leptin, insulin, glucose or urea (Kelly et al., 2010). Feed intake was also higher for animals with high RFI i.e. less efficient at converting feed energy. The phenotypic correlation between RFI and DMI ranged from 0.60 to 0.72, suggesting selection for lower RFI could decrease feed intake (Arthur et al., 2001; Nkrumah et al., 2007).

GWAS is a rapidly advancing technology, however in terms of the understanding of feed efficiency, using this technique is still in its infancy, with previous research findings providing only a very generic description of gene regions that are associated with the feed efficiency trait. Further development is crucial for this information to be used in practice.

2.6 Predicting feed efficiency

We have established that there is a need to improve feed utilisation in cattle to reduce environmental emissions. Dietary manipulation has somewhat contributed to increases in efficiency (e.g. Dewhurst et al., 1996; Merry et al., 2006; Brito et al., 2007; Bryant et al., 2010; Ellis et al., 2011; Cheng, 2012), however this is less of an option for free grazing animals. There is still much to understand in the underlying genetic control of feed efficiency and its components. The weakness is in the ability to collect large amounts of feed efficiency data to evaluate these genetic effects. There is a need to improve phenotypic (or genetic) predictors that would provide a low cost and simple sampling protocol for free grazing animals to collect large amounts of animal data. Success in identification of these markers has been limited; many that are moderately good predictors of feed efficiency have been evaluated in beef cattle and many still only account for approximately 20% of the variation in

efficiency (Table 2.2). Some markers of interest that are related to N efficiency are described below.

2.6.1 Metabolic markers

There has only been moderate success in identifying blood metabolites as potential indicators of feed efficiency. Urea, a liver metabolite, has been positively genetically and phenotypically correlated to FCE in beef heifers (Kelly et al., 2010; Table 2.2). A higher level of blood plasma proteins and aspartate amino transferase has also been found in cattle that have higher feed efficiency (Richardson et al., 2004), which may be due to a higher protein intake in higher RFI animals (Lush et al., 1991). The positive correlation between urea, FCE and DMI is likely to have a similar basis to that of urea and dietary N (Clarke et al., 2009).

Other blood metabolites that have been associated with feed efficiency are calpastatin (McDonagh et al., 2001), serum cortisol (Knott et al., 2008) and insulin-like growth factor-1 (IGF-1) (Richardson et al., 2002). Johnston (2007) found a high correlation between IGF-1 and RFI (0.39-0.56) and a high heritability (0.34-0.43); however this genetic correlation decreased with maturity. This may be useful in young animals for pre-screening in breeding selection, and would also be cost effective (Wood et al., 2002), but more recent findings have found no association between plasma IGF-1 and FCE or RFI (Kelly et al., 2010).

Molecular markers may also be a useful way to indicate feed efficiency in breeding animals e.g. in pigs, IGF-2 a paternally expressed QTL affecting muscle mass (Amarger et al., 2002) and melanocortin-4 receptor (MC4R) which mediates leptin circulation. Other potential markers with RFI are uncoupling protein 2 (UCP2), uncoupling protein 3 (UCP3), neuropeptide Y, and growth hormone (Sherman et al., 2008), targeted for their function in energy homeostasis, growth and intake, as well has having functions affecting metabolism and appetite.

Table 2.2 Literature values for potential markers of feed efficiency (or components of feed efficiency) and their predictive ability (r^2)

Reference	Animal description	*n*	Diet	Efficiency Measurement	Metabolite	r^2
Richardson et al. 2004	Steers	33	Feedlot ration	FCR, kg feed/kg gain	Albumin, g/L	0.32***
					Urinary 3-MH to creatinine ratio	0.23**
		17			Glucose, mmol/L	0.21*
					Aspartate aminotransferase, U/L	0.19*
		33		RFI, kg/day	Insulin, ng/mL	0.18*
					Cortisol, ng/mL	0.16*
		17			Aspartate aminotransferase, U/L	0.18*
					Creatinine, µmmol/L	0.20*
Kelly et al. 2010	Growing beef heifers	86	TMR	FCR, DMI/kg LWT gain	Leptin, ng/mL	0.23***
					Urea, mmol/L	0.18***
					Glucose:insulin	0.23*
Nkrumah et al. 2006	Steers	27	Concentrate	RFI, kg/day	Methane, L/kg of $BW^{0.75}$	0.19*
Hegarty et al. 2007	Angus steers	76	TMR	RFI (15d)	Methane, L/kg of $BW^{0.75}$	0.12**
Lancaster et al. 2008	Angus bulls and heifers	39	Roughage based	FCR, kg feed/kg gain	IGF-1	0.24*
Lancaster et al. 2008	Angus bulls and heifers	39	Roughage based	RFI, kg/day	IGF-1	0.16*
Johnston, 2007	Beef cattle			RFI, kg/d	IGF-1	0.15-0.31*
Nousianien et al. 2004	Dairy cows	306	Grass silage	g milk protein/kg N intake	MUN (g/d)	0.77***
	Dairy cows			Urine N, g/d	MUN (g/d)	0.79***

***P<0.001, **P<0.01, *P<0.05

2.6.2 Urinary N

Urinary N excretion is a major potential source of N pollution in dairy cows so its manipulation is vital for future improvements (Pacheco et al., 2010). The excretion of urine, milk and faecal N can account for up to 72% of N intake (Castillo et al., 2000). Forage with low dry matter (DM) increases the dilution of urine, decreases N concentration but increases urine volume. In addition, fermentability of these forages also alters the availability of nutrients from rumen digestion and this therefore affects urine N output (Pacheco et al., 2010). Urinary N is not reliable as a direct predictor of NUE because reducing urinary N does necessarily indicate improved feed efficiency, however it can still aid in identification of the variation of NUE within a herd. Creatinine or urine metabolomics using GCMS have also been investigated as possibilities for using urine as an indicator of N utilisation (Bertram et al., 2011).

2.6.3 Milk urea N

Milk urea N (MUN), a result of protein synthesis inefficiency (Stoop et al., 2007) is an inexpensive, non-invasive measure with potential to indirectly estimate NUE and identify dairy cows that are fed excessive N in the diet (Nousianien et al., 2004). Efficiency of dietary protein is highest when the protein supply in the diet matches rumen and tissue requirements. Excess N in either of these pathways is associated with higher amounts of absorbed urea, which results in lower NUE (Baker et al., 1995). A deviation from the optimum level 8.5 to 12 mg/dL (Linn and Raeth-Knight, 2007) generally indicates excess protein in the feed. Milk urea N is best used for an evaluation of a whole herd's nutritional status because there will be seasonal and breed variation between individuals. For this reason there is potential difficulty in grouping animals divergent for high and low MUN. There have been some promising results (e.g. Stoop et al., 2007), however it has also been associated with negative effects on production and fertility and a low heritability (Hossein-Zadeh and Ardalan, 2011). Nousanien et al. (2004) reported a strong relationship between milk urea and N utilisation; however this was mainly driven by diet (Table 2.2).

Milk urea nitrogen has been used to indicate the rumen efflux of crude protein to show N losses of rumen fermentation, but does not represent the efficiency with which absorbed protein is utilised (Hof et al., 1997). A strong correlation between MUN and blood urea N has also been reported, with both MUN and blood urea N being sensitive to changes in the supply of CP, RDP and RUP, but insensitive to the changes in amino acid balance (Roseler et al., 1993; Baker et al., 1995).

2.6.4 Breeding indexes

Breeding indexes are used to rank animals that are genetically more favourable for selection. The New Zealand animal evaluation system (New Zealand Animal Evaluation Limited (NZAEL), New Zealand) is used to rank animals that are most efficient at creating profit and high value replacements. It is calculated for bulls, cows and a whole herd basis for evaluating current season or expected performance. Breeding value (BV) ranks males and females for their genetic merit for individual traits, production value (PV) ranks females for their lifetime production ability, and lactation value (LV) ranks females for their current season production ability. The combination of the economic worth of important traits (e.g. milk fat, protein, milk volume, LWT, fertility, somatic cells and residual survival) gives an overall breeding worth (BW) value for the animal in NZ$. The Irish index system, economic breeding index (EBI) is a similar profit index that identifies the most valuable bulls and cows for replacement and breeding. It weights data from factors including milk production, fertility, calving, performance, beef carcass, maintenance and health to a single economic breeding value (Berry et al., 2007).

These indexes do not directly select for feed efficiency components or traits, however they do select for aspects such as increased milk protein and milk yield, which may indirectly select for increased efficiency. Using efficiency measurements in a ranking system may be useful; however the consequences or adverse effects of incorporating these traits would need evaluation over many generations and large numbers of animals to be beneficial.

There has been some repeatability in the literature for some markers for feed efficiency in beef cattle, but these have not been robust enough for implementation in breeding programmes. The gap in the literature is that aside from MUN in dairy cattle that is sparingly used as an indicator of N efficiency, there are currently no phenotype markers that serve as predictors of N efficiency. To find a marker that correlates to feed efficiency, it is important to first understand the metabolism of N through ruminants in order to target metabolites that are likely to be related.

2.7 Metabolism of nitrogen in ruminants

The supply of metabolisable protein (MP) (absorbed amino acids) depends on the supply of rumen degradable protein (RDP), the rate of microbial protein synthesis and digestion in the small intestine discussed previously (Section 2.4.1) The metabolism of amino acids is a complex process of recycling, catabolism, anabolism and involves and integration with urea synthesis and the ornithine cycle, and the hydrolysis, absorption and excretion of many compounds. Diet is the major influx of N which is released into the gastrointestinal tract; some of this protein is hydrolysed into free amino acids which join the pool of circulating amino acids in the body whilst the rest is excreted in faeces. There is substantial turnover of body protein and body proteins that are degraded return back to the pool of amino acids (Balter et al., 2006). In the liver, the main amino acid metabolism pathway is protein synthesis and the other is urea excretion via the ornithine-urea cycle (Sick et al., 1997) (Figure 2.2).

2.7.1 Ammonia and urea metabolism

Approximately 98% of N in the ruminant is in the form of protein and amino acids (Schoeller, 1999). Dairy cows excrete urinary N in two main forms; urea and ammonia, and some smaller components including guanine and amino acids (Wright, 1995). When estimating urinary losses of N, there are obligatory losses which include compounds containing N not used in protein turnover, and facultative losses including the excretion of urea (Kebreab et al., 2001). Ammonia is derived from

peripheral tissues and is the end product of amino catabolism and is also released from urea by micro-organisms in the digestive tract. Glutamine synthesis is an important process in ammonia detoxification to enable the dairy cow to excrete N in the form of urea (Balter et al., 2006). In this process, excess amino acids not required for maintenance and lactation are catabolised to ammonia which is toxic to the animal so is subsequently carried by glutamine for conversion to urea for excretion (Wright, 1995). This conversion allows urea to be retained in the body for longer periods of time without toxic effects (Wright, 1995). The catabolism of ammonia involves a two-step reaction (i) the transamination of amino acids to form glutamate (Wright, 1995), and (ii) the deamination of glutamate to ammonia ($NH4^+$) and α-ketoglutarate, a keto acid produced by deamination of glutamate, by glutamate dehydrogenase (GDH).

Transamination does not occur equally between all amino acids and as a result, the partitioning of amino acids varies (Macko et al., 1986). Differences in amino acid requirements depend on the animal's age, health and nutritional status (Young and El-Khoury, 1995) and the lactating cow also has an major additional route of N flux into milk (Schoeller, 1999).

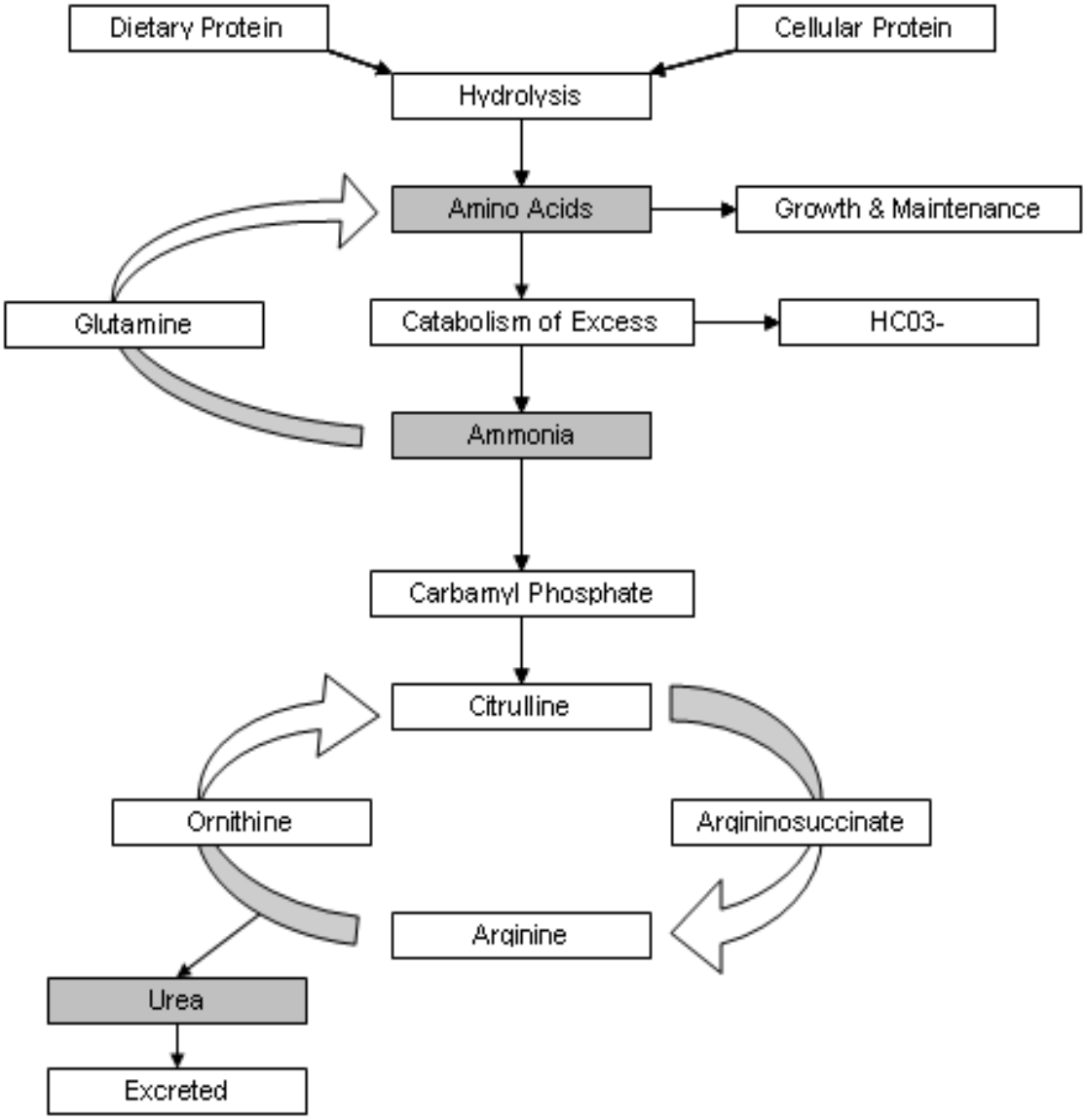


Figure 2.2 The main routes of N metabolism in mammals and recycling of urea (Adapted from Wright, 1995; Korf, 2006).

Urea is the end product of N metabolism. The ammonia after deamination of amino acids is toxic so it has to be converted to urea to be removed using the urea cycle in the liver. The urea cycle consists of a number of steps; the major steps consist of (1) transfer of a carbamoyl group from carbamoyl phosphate to ornithine to form citrulline, (2) enzymatic activation of citrulline by forming an intermediate molecule, which is transformed by the amino group of an aspartate residue to form arginiosuccinate, (3) arginiosuccinate is cleaved into fumarate and arginine and (4) arginine is cleaved to produce urea and ornithine, completing the cycle (wright, 2005).

The additional complexity to ruminant N metabolism is a result of the microflora in the rumen. Ammonia produced by these organisms supports protein synthesis, especially in animals fed on low protein diets where diet alone cannot sustain the demand for milk protein (Kay et al., 1980). Rumen fermentation also aids in other metabolism pathways, for example, urea produced in the liver is recycled and degraded to ammonia by the enzyme urease in the microbes (Church, 1975). Microbial cells in the rumen that utilise aspartic or glutamic acids channel amino acids directly to amino acid pools without deamination, whilst the rest are synthesised by transamination (Macko and Estep, 1984).

2.8 Stable isotope fractionation

The complex nature of N cycling in the ruminant means it is very difficult to find a phenotypic marker that can identify small differences in N metabolism in many different pathways. A novel marker based on stable isotope fractionation may offer a solution because it has been used extensively in archaeological and medical research to reconstruct diet composition of mammals and to quantify the flow of nutrients and biological pathways through animals (Weast, 1983; Sponheimer et al., 2003; Nardoto et al., 2006). Fractionation of isotopes results in changes to the ratio of heavy to light isotopes in a sample; in this case we are interested in the ratio of ^{15}N to ^{14}N (Robinson, 2001; Fuller et al., 2004; Robbins et al., 2005; Balter et al., 2006). Stable isotope techniques can measure trophic positions on food webs, sources through a

food chain, community structure and migratory behaviour (Wang et al., 2004) as well as evaluation of predator-prey relationships demonstrating energy flow through a complete ecosystem.

2.8.1 Nitrogen isotope fractionation concept and reactions

Nitrogen fractionation occurs in either kinetic or equilibrium reactions. During equilibrium reactions, the enriched or heavier isotope is concentrated in the tissue with a higher oxidation rate, resulting in depletion in other tissues, in a reversible system that is in equilibrium (Weast, 1983). Generally lighter isotope bonds are broken down more easily than bonds involving heavier isotopes so they react faster and produce more product which leaves greater amounts of the heavy isotope concentrated in the tissues. The magnitude of this fractionation depends on the reaction rate, the strength of the bonds, the reactant volume and anatomical and environmental variations (Kendall and Caldwell, 1998).

Kinetic isotope effects (KIE) are produced by the rate of reaction during a substitution of one molecule with its isotope (Cook, 1991). In the case of differences between two isotopes nitrogen-14 (^{14}N) and nitrogen-15 (^{15}N), the element has the same number of protons but different number of neutrons and the heavier isotope (^{15}N) will behave differently to its corresponding isotope. The heavier molecule has a lower zero point energy because more energy is needed to break the bonds, therefore higher bond dissociation energy and a slower reaction rate. The substitution of the isotopes are commonly explained by the replacement of hydrogen with deuterium which is the ratio of the rate constants kH/kD, as the KIE for this reaction is the largest because of the substantial change in mass of the atom during the reaction (Chapman, 2009). In comparison, KIE reactions for ^{14}N and ^{15}N are smaller because the percentage change in mass is smaller. KIE fall into two categories (i) primary, which occur from substitutions where the bond is broken and (ii) secondary, where KIE are from substitutions at a site other than where the bond is broken (Cook, 1991).

For naturally abundant isotopes, the magnitude of the isotope effect is explained by the following equation (Kendall and Caldwell, 1998);

Fractionation = (heavy to light ratio) product / (heavy to light ratio) reactant
When the result is greater than 1 the product is considered as 'enriched'. Units for fractionation are expressed by the ratio change from the heavy to light isotope using delta (δ) units;

$$\delta = (R\ (sample) - R\ (standard)) \times 1000 / R\ (standard),$$

where R is the heavy to light isotope ratio, and δ is delta per mil (molecules per thousand; 10 times the percent difference of the ratio of heavy to light isotope) (McKinney et al., 1950) or alternatively using ‰ (per mil). The enrichment factor is expressed by using the symbol Δ. In the case of enrichment of $\delta^{15}N$, $\Delta^{15}N$ = animal tissue $\delta^{15}N$ ─ diet $\delta^{15}N$.

2.8.2 Understanding fractionation pathways in biological systems

Predicting pathways of N fractionation is complex; there have been attempts to model isotope distributions to understand the combination of biological processes as an overall system (Fry, 2002). They have been frequently used to locate dietary N tracers and identify changes in open systems with continual biological shifts in multiple inputs and outputs, which require an assumption of steady-state balance (Schoeller, 1999). For dairy cows, lactation causes additional body stress so during this time they are in a state of non-balance which further complicates their biological shifts. Simulation models can be used to understand non steady-states, however the models are more complex (Hobbie et al., 1999). Mathematical models combined with fractionation values quantify components of a diet to the animal, and have become increasingly challenging as more pathways are discovered that interact in overall isotopic distribution (Koch and Phillips, 2002).

The most simple fractionation model of an organism is a steady state system with two compartments in balance with one output from each (Steele and Daniel, 1978); in the case of dietary N, this can be split into two simplified pathways through the ruminant, protein supply and non-protein N. The assumptions of the model assume the most likely site of fractionation is elimination pathways, leaving the body tissues relatively enriched in the heavier isotope (Schoeller, 1999). More recently researchers have developed more complex models to incorporate amino acid interactions, urea re-utilisation and synthesis of proteins by ruminal bacteria (Ponsard and Aerbuch, 1999).

A detailed model of isotope signatures by Balter (2006) presents the transfer of N through several biological pathways and prediction of ^{15}N ratios of reservoirs by using animal weight, dietary N intake and time (Figure 2.3). The model describes $\Delta^{15}N$ of body tissues relative to the diet as the result of two pathways of fractionation competing with transamination and N transfer during the urea cycle; cycling of N is produced through urea synthesis, hydrolysis and excretion and the synthesis of urea by the Krebs-Henseleit cycle. Using this model, fractionation values are estimated during transamination and between various fluxes e.g. urea hydrolysis (Macko et al., 1986), some of which were taken from literature e.g. values from Silfer et al. (1992) and Hermes et al. (1985).

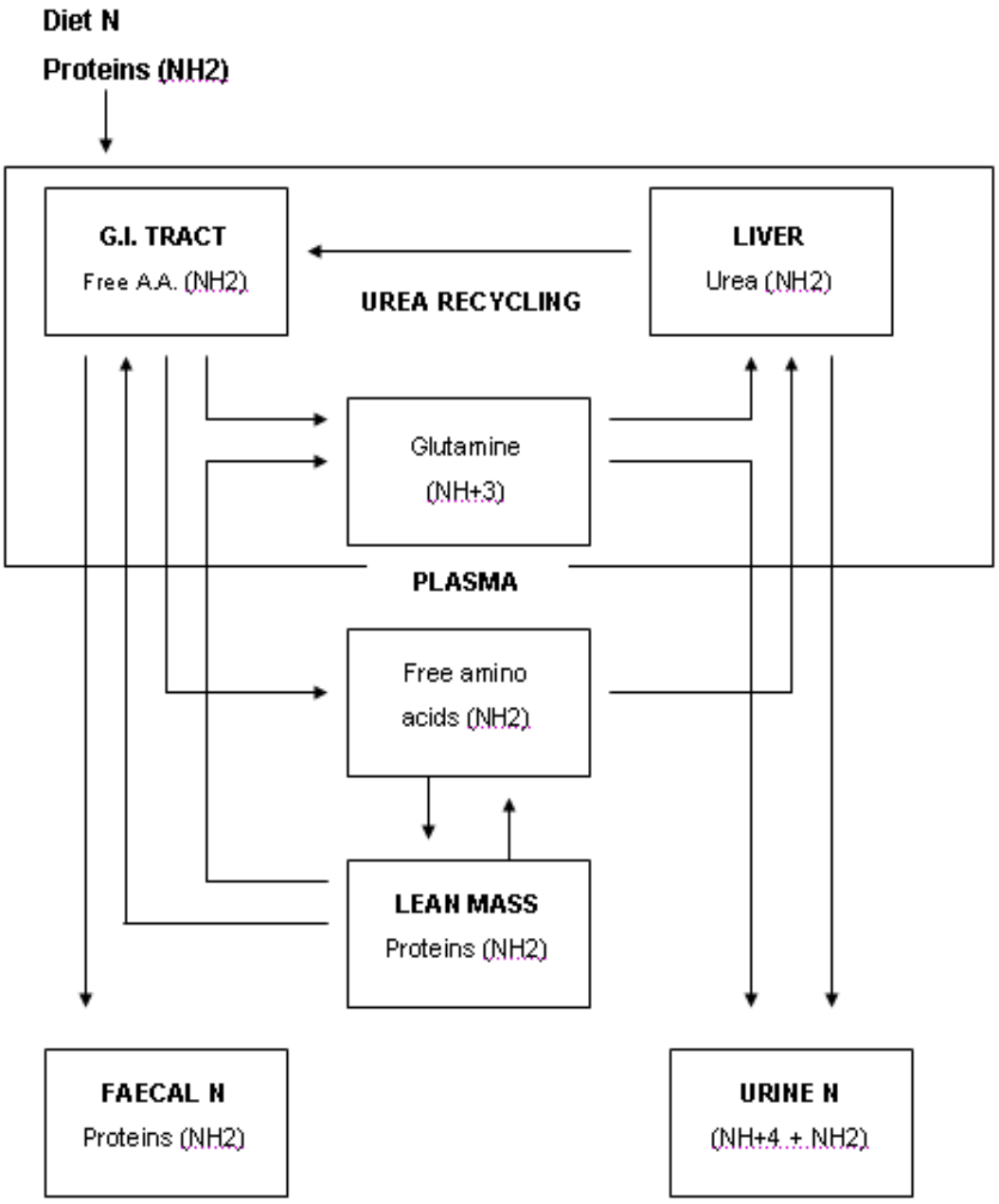


Figure 2.3 Fluxes of N and associated pathways of excretion and recycling (Adapted from Balter, 2006).

The model evaluates the effects of variation in body mass on the time required for different reservoirs of N to reach isotopic steady state (or equilibrium) (Balter, 2006). Without the ability to measure real-time fluxes inside the ruminant, these models are a good way to schematically understand N metabolism and fractionation in animals but further research is needed to understand the processes which account for the $\Delta^{15}N$ factors in an organism and during the process of N metabolism (Balter, 2006).

2.8.3 Isotope fractionation in nitrogen metabolism pathways

Most fractionation is likely to occur in liver tissues, and to a lesser extent tissues such as the small intestine. During the process of transamination and deamination in urea synthesis ^{14}N from the feed is preferentially used due to its lighter mass, resulting in a depletion of ^{15}N in urea and ammonia formed in the liver, while proteins are enriched compared to the amino acids of the free metabolic pool (Sick et al., 1997). This process, termed isotope ratio disproportionation, is a common occurrence in amino acid metabolism in several tissues (Sick et al., 1990). Transamination following the glutamine cycle results in a 10% difference in ^{15}N between the newly formed asparate and the remaining glutamate in the tissue (Macko et al., 1986). The change in the oxidation state between ammonia bound to glutamine has also been suggested to cause an equilibrium fractionation of N.

The rate of deamination varies among amino acids (Scheifinger et al., 1976), so amino acids in the diet that are more easily deaminated will have higher fractionation rates (Macko et al., 1987). While dietary protein is the predominant driver in the rate of fractionation, dietary supply is not solely responsible (Young and Pellet, 1988). It has also been hypothesised that fractionation of N isotopes increases with increasing protein in the diet and decreasing ratio of carbon to N, so animals on a higher tropic level should have higher fractionation rates than ones below it (Robbins et al., 2005). The complex pathways of N fractionation in liver tissues have been somewhat understood by theoretical modelling and studies on small mammals (Macko et al., 1986; Sick et al., 1990), but the process of fractionation in the ruminant is still largely unknown.

2.8.4 Nitrogen isotope fractionation in mammals

Nitrogen fractionation has been widely used to study several mammal species such as bears and their hibernating behaviours (Hobson et al., 2000), pig tissues (Nardoto et al., 2006), llamas (Sponheimer et al., 2003), birds (Robbins et al., 2005), sheep (Sutoh et al., 1993) and lactating humans (Fuller et al., 2004) and also used as markers for erythrocytes (Read et al., 1974). There has been a rapid growth in the use of these methods because they are inexpensive and easy to measure, but understanding the link to metabolism events is difficult (Caut et al., 2010). In comparison to ruminants, stable isotope ratios have been used with plants for decades (Gannes et al., 1997) to trace plant N sources, flow and NUE (Handley and Raven, 1992).

It has been consistently found that animal tissues are more enriched in $\delta^{15}N$ than their diet (e.g. Steele and Daniel, 1978; Ambrose and De Niro, 1986; Sutoh et al., 1993; Cheng and Dewhurst, 2009; Cheng et al., 2010) because of the preferential utilisation of ^{14}N as it is lighter in mass so uses less energy (Macko et al., 1986; Macko et al., 1987). Average enrichment is 3‰ units heavier in animal tissues compared to their diet (Sponheimer et al., 2003) but this can vary. Caut et al. (2008) found large variations in the magnitude of enrichment in Sprague-Dawley rats, and Minagawa and Wada (1984) found a range between 1.3‰ and 5.3‰ in marine and fresh water animals. In addition, not all animals follow the theory of enrichment; Sponheimer et al. (2003) found no significant differences between $\delta^{15}N$ fluxes in urine and faeces in llamas on different diets.

2.8.5 Nitrogen isotope fractionation research in ruminants

Since the first measurements of N fractionation in cattle by Steele and Daniel (1978) there have been several studies with different ruminants that have confirmed the theory of tissue enrichment in N isotopic fractionation (Table 2.3). High $\Delta^{15}N$ is generally seen in animals at maintenance or with low growth rate (e.g. 6.94 for mature wethers; Sutoh et al., 1993) and lower $\Delta^{15}N$ for young developing animals i.e. neonates (1.5 for moose calves less than 3 months old fed milk; Jenkins et al., 2001). Studies have also considered fractionation by ruminal bacteria, as it is a complex site of protein metabolism. Nitrogen fractionation occurs during the metabolism of ammonia when rumen microflora ferment glucose and cellulose and does not occur during metabolism of amino acids, deamination and bacterial lysis (Wattiaux and Reed, 1995). Macko and Estep (1984) identified fractionation during the stepwise pathway of urea synthesis, when the glutamate synthesising enzyme combines with ammonia to form glutamate. The fractionation effect of the absorption of ammonia from the rumen and urea synthesis on N partitioning is unknown. Nitrogen isotopes in microbial bacteria which are cultured in ammonium bicarbonate have the potential to be used to explore N metabolism because of the separation of isotopes during ammonia synthesis (Wattiaux and Reed, 1995). The excreted N in ammonia and urea is lighter than tissue and dietary protein (Steele and Daniel, 1978) and enrichment of these tissues depends on various factors of protein metabolism and cycling in the rumen system (Gannes et al., 1997).

Nitrogen fractionation increases as dietary protein quality decreases. When excess protein is supplied in the diet, milk N decreases and urine N increases, and during this process no fractionation occurs (Wattiaux and Reed, 1995). Microbial protein synthesis from amino acids causes no fractionation of N, however in contrast there is an increase in milk N and reduction in urine N (Wattiaux and Reed, 1995). Biological reactions in the ruminant are generally kinetic e.g. the deamination of amino acids and active transport, and less so equilibrium reactions e.g. diffusion of molecules such as ammonia (Wattiaux and Reed, 1995).

2.8.5.1 Sites of fractionation

Much of the knowledge of specific sites of fractionation are unknown. The differential metabolism of N isotopes is related to NUE and dietary N intake, through the absorption of ammonia or digestion of protein in the small intestine, and is also related to animal variation. The basis of the theory that NUE may be related to N isotope fractionation is that during the metabolism of dietary protein, the nitrogen pathways leading to milk and urine respectively causes a fractionation of N isotopes. The more efficient an animal is a converting dietary protein into milk protein, the lower the isotopic effect as there is less conversion of excess N to urea.

The main site of fractionation occurs in the liver during transamination and deamination in urea synthesis (Macko et al., 1987). N fractionation also occurs during metabolism of ammonia during microbial fermentation of glucose and cellulose in the rumen, and when glutamate synthesising enzyme combines with ammonia to form glutamate. In the case of increasing dietary N, an increasing proportion of N is directed to urine so the fractionation rate increases (causing a higher isotopic signature), because higher amounts of ammonia and urea are formed in the urea cycle. If there is more urea synthesis from catabolised amino acids (and potentially ammonia) there will be more enrichment of body tissues and depletion of urea. Therefore whether the urea synthesis is due to the catabolism of amino acids absorbed in excess of requirements, or excess ammonia absorption (both due to higher N intakes), tissue enrichment of $\delta^{15}N$ would be higher relative to the diet.

Table 2.3 Literature values for N isotopic fractionation in ruminants ($\Delta^{15}N$; $\delta^{15}N$ in blood minus $\delta^{15}N$ in the diet)

Reference	Physiological state	Animal description	Diet	$\Delta^{15}N$	Sample type
Steele and Daniel, 1978	Growing/mature	Angus steers	Ryegrass/white clover silage	4.2	blood
Sponheimer et al. 2003, ‡	Growing/mature	Cattle	Lucerne hay	4	blood
Koyama et al. 1985	Growing/mature	Beef cattle	Rice straw	3.8	blood
Sponheimer et al. 2003, ‡	Growing/mature	Goats	Lucerne hay	4.7	plasma
Sutoh et al. 1989	Mature	Goats, 2 year old females	Lucerne hay cubes	4.8	plasma
Sutoh et al. 1993	Mature	Wethers, 4 years old	Lucerne hay cubes	6.94	plasma protein
Darr and Hewitt, 2008	Mature	Deer, 2 year old males	Lucerne	5.67	serum
Darr and Hewitt, 2008	Mature	Deer, 2 year old males	Lucerne/maize (3:2)	6.2	serum
Koyama et al. 1985	Lactating	Beef cows, milking	Pasture	2.59	blood
Sutoh et al. 1993	Lactating	Lactating dairy cows	Forage/concentrates (1:1)	2.37	plasma
Cheng et al. 2013a	Lactating	Lactating dairy cows	Grass	3.19	plasma
Jenkins et al. 2001, ‡	Lactating	Sheep, early lactation	Hay	5.1	plasma
Jenkins et al. 2001, ‡	Lactating	Sheep, early lactation		4.5	plasma
Jenkins et al. 2001	Neonate	Deer calves: 12-14 days old	Milk	1.9	plasma
Jenkins et al. 2001	Neonate	Lambs: 12-14 days old	Milk	3	plasma
Jenkins et al. 2001	Neonate	Moose calves < 3 months old	Milk	1.5	plasma

‡ also unpublished data cited by Robbins et al. (2005)

Few studies have been able to consider the time course of incorporation and depletion of $\delta^{15}N$ in tissues during the processes of fractionation because we are not able to sample within the organism at desired time points (Bond and Barret, 1993). However, Hwang et al. (2007) investigated $\delta^{15}N$ and the digestive physiology of small mammals at different locations in the gastrointestinal tract and showed a consistent enrichment of $\delta^{15}N$ in the stomach and intestines, and depletion in faeces (although more enriched than other organs). In pig tissues, Hare et al. (1991) studied fractionation on a molecular level for individual non-essential amino acids and, except for threonine, all amino acids had higher $\delta^{15}N$ relative to those in the diet. In particular, glutamate was most enriched relative to the diet.

It is generally accepted that using equilibrium shifts for a set time period give fair estimates of fractionation values for that animal at that point in its lifetime (Caut et al., 2010) but in order to ultimately gain 'true' discrimination values, Auerswald et al. (2010) argued that lifelong experiments are needed detailing nutrient flow, parentage and feeding habits. Comparisons between non-ruminant and herbivorous animals and ruminants would be useful to determine the extent that the rumen provides for additional N recycling (Robbins et al., 2005).

Studies on N fractionation in dairy cattle by Cheng and Dewhurst (2009) have shown consistency in the idea of enrichment of $\delta^{15}N$ in milk protein and depletion of $\delta^{15}N$ milk non-protein N relative to non-separated milk. In a study using sheep, $\delta^{15}N$ values of faeces were 3‰ higher relative to the diet as a result of endogenous body tissue protein excretion, and urine values were 0.7‰ higher than plasma urea, the main precursor of urinary N (Sutoh et al., 1993). In addition, muscle and plasma $\Delta^{15}N$ have been useful in predicting NUE in non-lactating sheep (Cheng et al., 2013b). Cheng et al. (2013a) found a significant relationship for milk $\Delta^{15}N$ as a proxy for NUE in a study on Holstein-Friesians cows, however found that the N fractionation approach may be unsuited to studies where there are diets containing differing levels of ammonia N (and so RDP) (Cheng et al., 2011; 2013a), since there is also a N isotope fractionation when ammonia N is incorporated in bacterial protein (Wattiaux and Reed, 1995).

Recent research and a greater understanding of stable isotope fractionation in ruminants have provided an opportunity to investigate this process as a predictor of N efficiency in cattle. It is a simple and inexpensive technique which could be used to measure efficiency in large numbers of animals. If successful, this can be used to assess efficiency in either a current season or point of time basis, or can be further investigated for heritability and genetic factors that could potentially be used in predicting progeny efficiency outcomes from parents.

CHAPTER 3

Comparison of nitrogen-use efficiency and energy conversion efficiency measured in Holstein-Friesian cows over an entire lactation cycle

3.1 Introduction

In this chapter the reliability of using NUE as a measurement for feed efficiency in dairy cattle was evaluated. Over the last two decades, there has been an increased focus on FCE in ruminant livestock (Archer et al., 1999; Kelly et al., 2010; Basarab et al., 2013) because feed represents a high proportion of input costs, and cattle are particularly inefficient at converting feed nutrients to products (Powell et al., 2013). Genetic selection to improve production has resulted in increased feed efficiency, particularly through a dilution of maintenance costs (McNamara, 2012). However, direct selection for feed efficiency has been slow in ruminants (Pryce et al., 2007); because of the difficulty in measuring feed intakes for large numbers of breeding stock. Whilst there has been noticeable success in breeding for FCE in other species (e.g. poultry; Case et al., 2012), difficulties in accounting for the partitioning of nutrients to milk production in dairy cows have also restricted progress.

There are three main sources of genetic variation in FCE, namely changes in body reserves, 'dilution of maintenance' effects, and differences in the efficiency of metabolic functions. Data are particularly difficult to interpret in dairy cows because of the mobilization and replenishment of body reserves that occurs during lactation and late gestation (Madhav et al., 1997; Friggens et al., 2004; Prendiville et al., 2009; Williams et al., 2013). The quantity of body protein that is mobilized and replenished appears to be much less than body fat (Smith and McNamara, 1990; Madhav et al., 1996). The pattern of change across the lactation cycle appears similar and net changes over an entire lactation cycle are small (Andrew et al., 1994; Komaragiri and Erdman, 1997; Moorby et al., 2002), so the contribution of body reserves to the variation in overall efficiency is likely to be small, except if measured at specific time points. Dilution of maintenance effects are largely driven by differences in milk

production, with maintenance costs for energy and protein making up a smaller proportion of total requirements for higher yielding cows (Khan et al., 2013).

Baldwin (1968) suggested a high (83%) and consistent efficiency of nutrient use for milk synthesis, and this was confirmed by Onken et al. (2011) in a modelling study with more recent data. This means that the main variation in metabolic pathways relates to maintenance processes, including basal activity, service functions and cell maintenance. Ion transport represents 30-40% of maintenance energy (ATP) requirements, and basal activities such as walking, eating and ruminating also account for approximately 15% of maintenance energy requirements (Baldwin et al., 1980; Arthur et al., 2001). Turnover of body protein is the other important maintenance process, and requires both ATP and amino acids.

The ultimate objective of this chapter was the identification of differences between animals in feed efficiency, whether these relate to genetics or management history. There has been a lot of research on NUE in cattle, but much of this has focused on diet effects, including the effects of N intake, the metabolisability of protein, and the amino acid profile of MP (Bell et al., 2000). In this study, we looked at the relationship between two estimates of FCE, a method based on the efficiency of metabolisable energy (ME) intake; energy conversion efficiency (ECE), measured as MJ milk energy produced per MJ ME of intake, compared to NUE measured as g milk N (N)/g total N intake over an entire year of recording of individual feed intake and milk production. The objective of this analysis was to compare NUE and ECE across a range of variation among individual animals rather than diet differences.

3.2 Material and methods

3.2.1 Experimental data

Data from 38 Holstein-Friesian heifers that were in their first lactation, and which data from an experiment has been published previously (Dewhurst et al., 2002; Moorby et al., 2002) were used for this study. All cows were initially housed in a free stall barn and fed the same diet during a 5 week period, then allocated randomly within age groups to two different dietary treatments.There was a 2 × 2 arrangement of treatments with animals having calved for the first time at either 2 or 3 years of age and allocated either 2 or 7 kg per day of concentrates (concentrate A; see Dewhurst et al. (2002) for full ingredients), plus *ad lib* ryegrass silage in the second half of their first lactation. All animals received the same dietary regime during the short dry period (6 weeks) between lactations where the diet consisted of *ad libitum* grass silage and barley straw (average 37% straw on an oven-DM basis) plus mineral and vitamin supplements (120 g/d). For the second lactation all cows were given the same standard diet comprising 8 kg/cow/d concentrate (concentrate B; see Dewhurst et al. (2002) for full ingredients) with *ad libitum* access to ryegrass silage. Concentrate B was reduced to 5 kg/cow/d after 120 days in milk.

Recordings were made over 12 months, from approximately the middle of the first lactation to approximately the middle of the second. Due to differences in timing of calving and drying off dates (the standard deviation for the date of second calving was 25 days), recordings in the first and second lactations both spanned 30 calendar weeks. There were 18 weeks of complete data (all cows) for the end of the first lactation and 18 weeks of complete data for the start of the second lactation, so all cows completed a total of 36 weeks of measurements. Intake and milk production information was handled as weekly means. Fresh forage was offered through Calan gates (American Calan Inc., Northwood, USA) each day at 10% in excess of previous consumption and refusals were recorded three times weekly. Feed was sampled each week for chemical analysis. Procedures for handling and analysing samples of feeds were described by Dewhurst et al. (1999), apart from N analysis,

which was performed on a Leco Nitrogen Determinator (model FP-428; Leco Corporation, St Joseph, MI). Weekly am and pm milk samples were analysed for fat, protein and lactose content (Milkoscan 605, Foss Electric, Denmark).

3.2.2 Energy Conversion Efficiency calculation

Using models to represent animal metabolism functions will always have limitations as the efficiency of energy use can vary up to 100% between individuals. However, there is confidence in analysing the comparison of NUE and ECE as these are both essentially components of the same measurement of feed efficiency. The models used well sourced equations and intake was recorded which makes efficiency estimates more accurate. As the model was analysed over the entire lactation it is a good representation of the influences of the fluctuation of milk energy on ECE equations. The ECE equations were based on milk energy equations from McDonald et al. (2002) which were improvements from the original equations in ARC (1980) that calculated milk energy from mainly just milk fat.

Energy conversion efficiency was calculated weekly (pooling of 7 days of data) for each cow as milk energy output (MJ/d) divided by ME intake (MJ/d). Milk energy (MJ/d) was calculated by multiplying milk energy (MJ/kg) by milk yield (kg/d) using the following equation taken from McDonald et al. (2002):

Milk energy (MJ/kg) = 0.0384 × fat (g/kg) + 0.0223 × protein (g/kg) + 0.0199 × lactose (g/kg) – 0.108.

Concentrate ME density (MJ/kg DM) was provided by the supplier. Forage ME was calculated using the following equation from Givens et al. (1989) based on NDF content. NDF was determined by methods described in Dewhurst et al. (2000):

ME (MJ/kg DM) = 14.91 – 0.0093 × NDF (g/kg DM).

3.2.3 Nitrogen-use efficiency calculation

Milk nitrogen (g/d) was calculated by dividing total milk protein (g/d) by conversion factor 6.38. Nitrogen-use efficiency was described as milk N (g/d)/ N intake (g/d). The calculation used recorded forage intakes and concentrate allocations for each animal, coupled with data from feed analyses, as well as recorded milk yields and milk composition data.

3.2.4 Statistical analysis

All statistical analyses were conducted using the Genstat for Windows statistical package (13th Edition; VSN International, 2010). The repeated measures analysis of variance (ANOVA) used the final 18 weeks of lactation 1, for which complete data were available, and 18 weeks of complete data from the beginning of lactation 2. Linear regression analyses were conducted to establish the relationships between ECE and NUE, milk N and NUE, and between milk energy and ECE independent of the treatment groups.

Weekly values for NUE and ECE for each cow were compared using a series of Pearson correlation analyses. An initial analysis of variance to identify major effects found an effect of concentrate level on ECE, and a concentrate level × age interaction effect for both ECE and NUE. The effects were then included into the analysis of variance for repeated measures (Genstat PROCEDURE Repmeas) that also included the fixed effects of cow and time (week of lactation). The goodness of fit of the variance-covariance structure used for fitting the data was a uniform covariance structure (compound symmetry) matrix.

3.3 Results

Previous analysis of milk production data (Dewhurst et al., 2002) presented data separately for lactations 1 and 2. This analysis presents a re-analysis of data across the whole experiment (excluding the dry period as cows were not lactating so efficiency calculations were not possible). Results for feed intake, milk production and composition, body weight, BCS and efficiencies for the entire 12 months duration of the experiment are presented in Table 3.1.

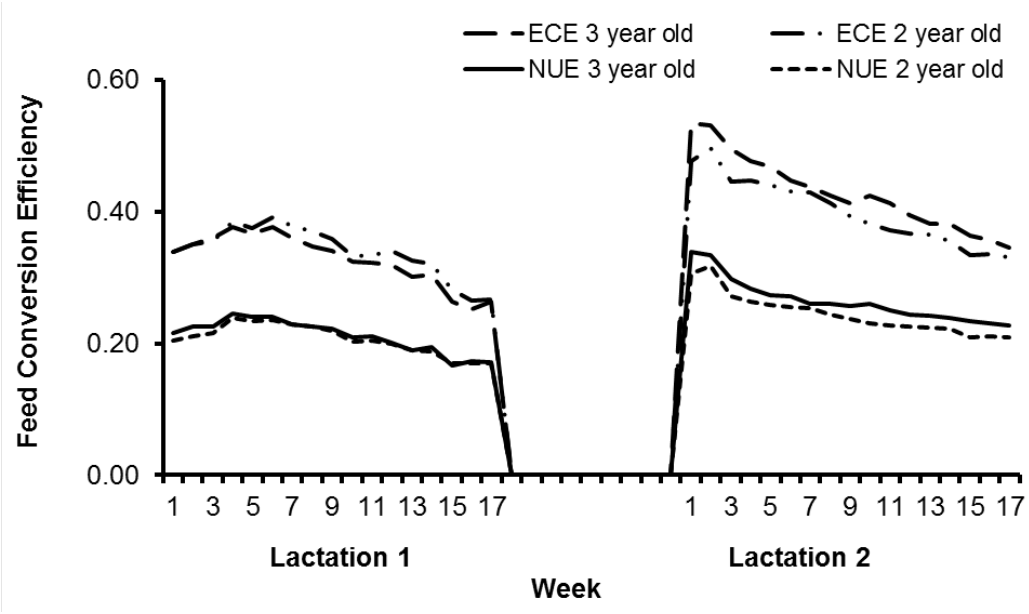
Table 3.1 Effects of treatments on feed intake, body composition and milk production for the four treatment groups over the entire experimental period

	Age at first calving					Significance			
	2 yr		3yr		s.e.d[a] (Interaction effect)				
Level of feeding:	Low	High	Low	High		Week	Age	Concentrate Level	Interaction
LWT, kg	645	596	669	656	24.9	***	*	†	*
DMI, kg/d	11.9	12.8	13.1	13.4	0.46	***	*	†	*
N intake, g/d	387	425	408	433	11.4	***	NS	***	**
BCS (Loin)	2.78	2.43	2.52	2.88	1.04	***	NS	NS	NS
BCS (Tail)	2.88	2.58	2.67	3.02	1.06	***	NS	NS	NS
Milk yield, kg	14.3	17.8	17.0	17.7	1.16	***	NS	*	*
Milk energy, MJ/d	49.7	60.7	58.4	60.4	3.88	***	NS	*	*
Milk fat, %	4.07	4.09	4.11	4.15	0.20	***	NS	NS	NS
Milk fat, g/day	660	808	784	811	56.3	***	NS	*	*
Milk protein, %	3.02	3.00	3.03	3.07	0.09	***	NS	NS	NS
Milk protein, g/day	488	595	577	595	34.6	***	NS	*	**
Milk lactose, %	4.08	4.15	4.07	4.04	0.10	***	NS	NS	NS
Milk lactose, g/day	675	834	786	805	57.6	***	NS	*	*
ECE, MJ/MJ	0.289	0.332	0.323	0.322	0.018	***	NS	†	†
NUE, g/g	0.182	0.204	0.202	0.199	0.010	***	NS	NS	†

[a]Standard error of the difference, †P < 0.1, *P < 0.05, **P < 0.01, ***P < 0.001, NS = not significant.

Energy conversion efficiencies and NUE for animals that first calved at 2 or 3 years old and received 2 or 7kg of concentrate in the second half of the first lactation are shown in Figures 3.1 (a) and (b) respectively. Mean efficiencies for the final 18 weeks of lactation 1 were: 0.20 (s.d. = 0.027) g/g for NUE and 0.33 (s.d. = 0.046) MJ/MJ for ECE. Corresponding mean values for the first 18 weeks of the second lactation were significantly higher than for the final 18 weeks of the first lactation ($P<0.001$); 0.24 (s.d. = 0.038) g/g for NUE and 0.39 (s.d. = 0.070) MJ/MJ for ECE. Despite the differences between lactations, there was also consistent between-animal variation, with some cows maintaining higher (or lower) efficiency values throughout the experiment. Average LWT gain over the 12 month period was 27.7 kg (s.d. = 52.7), which accounted for only 1.7% of average daily ME intake and explained just 9% ($P>0.05$) of the variation in the relationship between ECE and NUE.

(a)



(b)

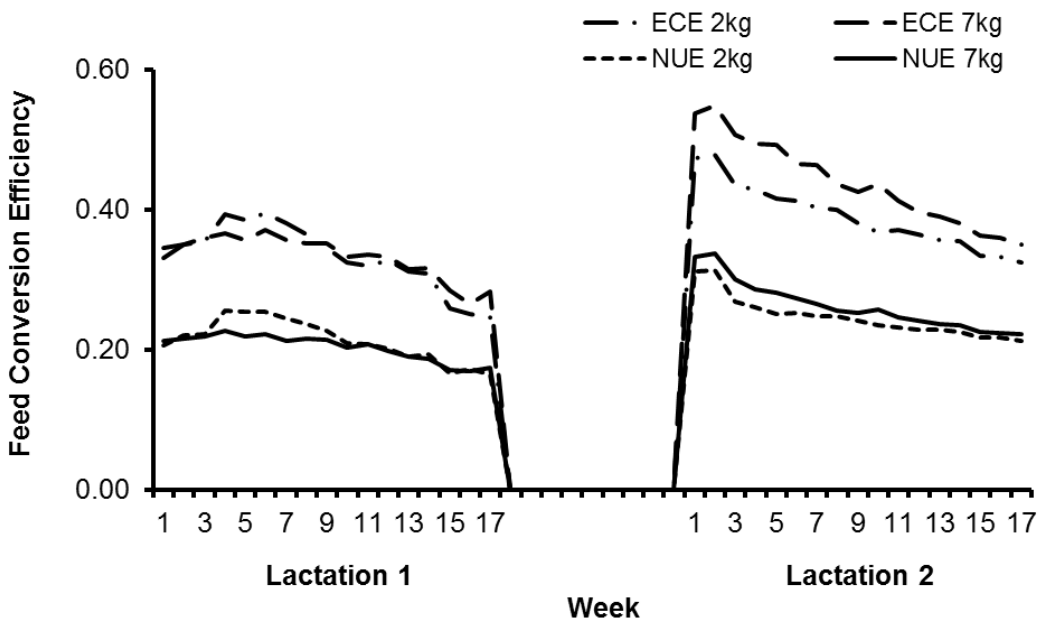


Figure 3.1 (a-b) Comparison of energy conversion efficiency (ECE; MJ/MJ) and NUE (g/g) over an entire lactation cycle from the middle of the first lactation to the middle of the second lactation for (a) animals that calved at either 2 or 3 years old and (b) animals that received either 2 or 7kg concentrate during the second half of the first lactation.

The results of the repeated measures analysis of variance are presented in Table 3.2. There were highly significant effects of time (week of lactation) on both NUE and ECE. Treatment effects on efficiencies varied over the entire experiment (Table 3.2) and between the lactations. The s.e.m. and CV% from the repeated measures analyses of variance were lower for models using NUE rather than ECE, for both lactation periods.

Table 3.2 Statistical significance, s.e.m. and coefficients of variation for treatment effects on energy conversion efficiency (ECE; MJ/MJ) and N-use efficiency (NUE; g/g)

	Measurement[a]			
	$ECE^{(1)}$	$ECE^{(2)}$	$NUE^{(1)}$	$NUE^{(2)}$
Significance				
Age.conc	NS	NS	NS	*
Time	***	***	***	***
Time.age.conc	**	NS	***	NS
s.e.m.				
Time	0.004	0.008	0.002	0.004
Age.conc	0.018	0.026	0.010	0.012
Time.age.conc	0.021	0.031	0.011	0.012
Subject	0.048	0.069	0.026	0.032
Subject.time	0.026	0.046	0.014	0.024
CV[b]				
Subject CV%	14.30	16.90	12.40	12.80
Subject.time CV%	7.90	11.30	6.90	9.40

[a] Measurement: ECE (1) = Milk Energy / ME intake (MJ/MJ) in the second half of the first lactation; ECE (2) = Milk Energy / ME intake (MJ/MJ) in the first half of the second lactation; NUE (1) = Milk N / N intake (g/g) in the second half of the first lactation; NUE (2) = Milk N / N intake (g/g) in the first half of the second lactation. Age = 2 or 3 years old at first calving, conc = concentrates; 2 or 7 kg/d of concentrates in the second half of the first lactation.

[b] Coefficient of variation.

†P < 0.1, *P < 0.05, **P < 0.01, ***P < 0.001

3.3.1 Correlations between ECE, NUE and milk production

There were highly significant relationships between NUE and ECE for the average of both lactation periods (r^2 = 0.90; P<0.001; Figure 3.2), and separate lactation periods. There were similar patterns of change for both efficiency measures across the different stages of lactation. Energy conversion efficiency estimates were higher than NUE across both periods and the correlation between the two measurements decreased especially the last 3 weeks of lactation 1 and during the first part of lactation 2 when changes in body reserves were largest.

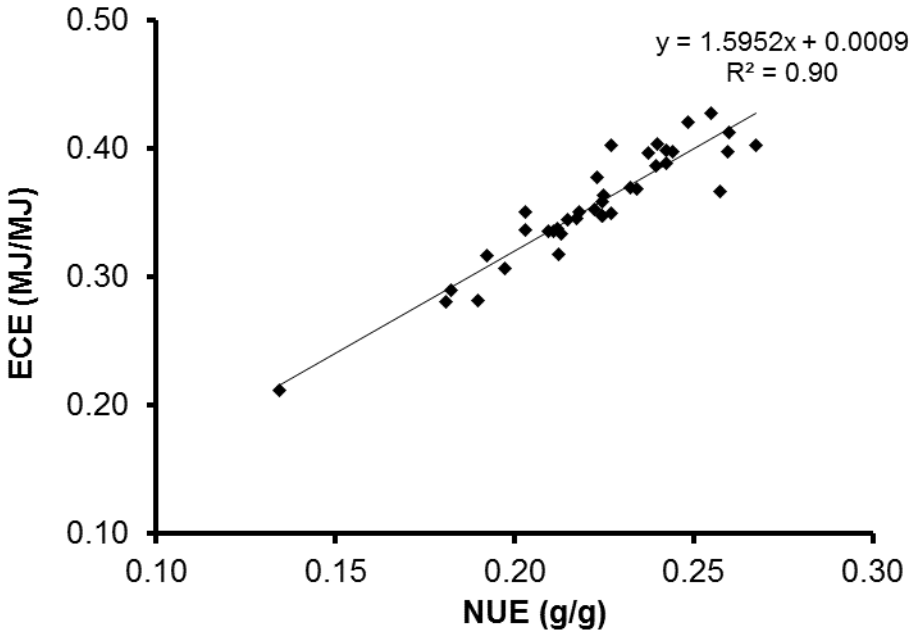
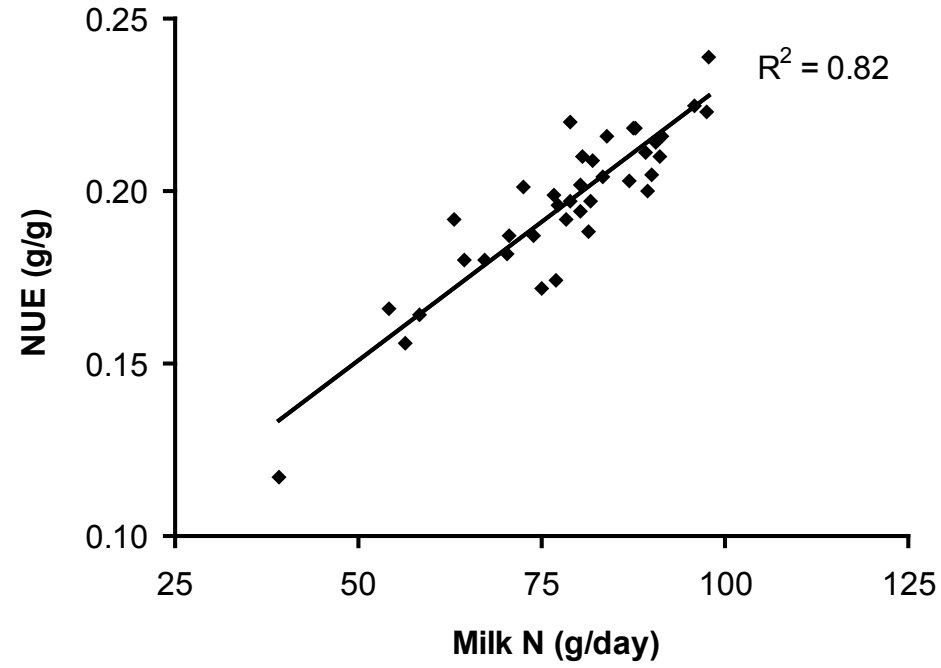


Figure 3.2 Relationship between energy conversion efficiency (ECE; MJ/MJ) and nitrogen-use efficiency (NUE; g/g) averaged over the 12 month period.

There was a highly significant relationship between milk yield (kg/d) and NUE (r^2 = 0.82; residual s.d. = 0.010) and ECE (r^2 = 0.80; residual s.d. = 0.018). There were weak, but significant (P<0.05) relationships between LWT and both NUE (r^2 = 0.13; residual s.d. = 0.02) and ECE (r^2 = 0.13; residual s.d. = 0.04). There was a weak, but significant (P<0.01) relationship between DMI and both NUE (r^2 = 0.17; residual s.d. = 0.02) and ECE (r^2 = 0.17; residual s.d. = 0.04). There were also weak, but significant (P<0.01) relationships, between ME intake (MJ/d) and N intake (g/d) and both NUE (r^2 = 0.20; residual s.d. = 0.02, and r^2 = 0.23; residual s.d. = 0.04) and ECE (r^2 = 0.20; residual s.d. = 0.02, and r^2 = 0.25; residual s.d. = 0.04) respectively.

Figure 3.3 (a) and (b) shows the relationships between milk output (milk N or milk energy) and feed efficiency (NUE or ECE).

(a)



(b)

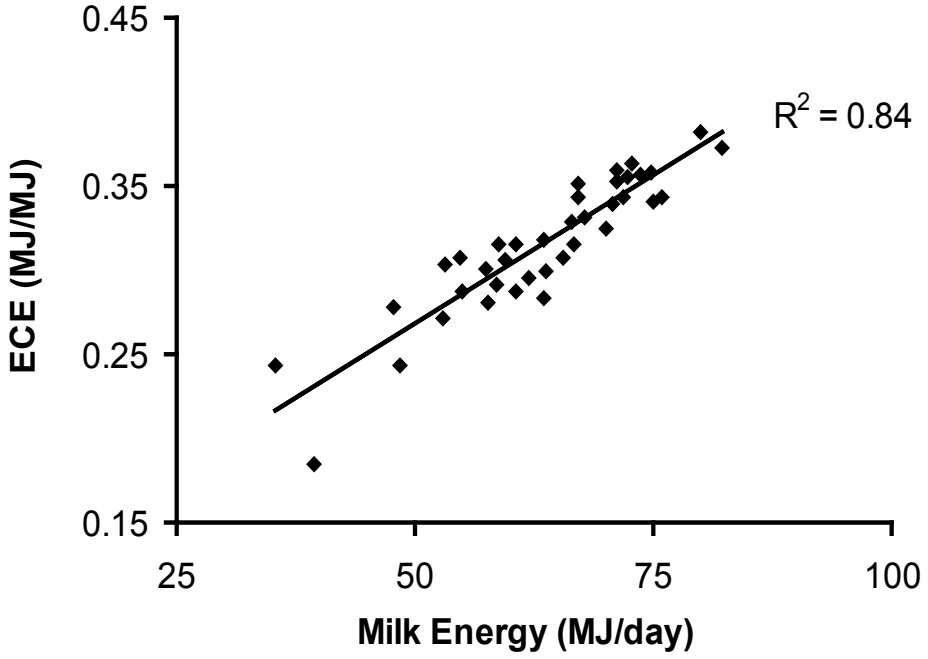


Figure 3.3 (a-b) Effects of level of production (milk energy MJ/d and milk N g/d) on feed efficiency, expressed as nitrogen-use efficiency (NUE; g/g) or energy conversion efficiency (ECE; MJ/MJ) across the entire experiment.

In each case, there were highly significant increases in efficiency with increasing production level (equations 1 and 2);

NUE (g/g) = 0.071 (s.e. = 0.0101) + 0.0016 (s.e. = 0.000127) × milk N (g/d)
n = 38; r^2 = 0.82; P<0.001; residual s.d. = 0.00985 (1)

ECE (MJ/MJ) = 0.086 (s.e. = 0.0153) + 0.0040 (s.e. = 0.000265) × milk energy (MJ/d)
n = 38; r^2 = 0.84; P<0.001; residual s.d. = 0.0150 (2)

3.4 Discussion

There is considerable variation in the efficiency of conversion of nutrients to milk among animals, even in groups of animals offered the same diet (Davey et al., 1983). Across the entire 12-month period of this study, the efficiency of conversion of dietary ME into milk energy for individuals ranged from 0.19 to 0.38 MJ/MJ and efficiency of conversion of dietary nitrogen into milk nitrogen ranged from 0.12 to 0.29 g/g.

3.4.1 Relationship between ECE and NUE

There are three main sources of variation in the relationship between ECE and NUE: changes in body reserves, 'dilution of maintenance' and differences in metabolic functions. Amongst the animal factors contributing to ECE and NUE, there are a number that are common to both pathways, whilst others are distinctive for energy- or amino acid-metabolism. Amino acid metabolism is a sub-set of overall energy metabolism, with absorbed amino acids representing around 15 to 30% of absorbed nutrients on an energy basis, and there are many amino acids that can be used for energy supply. At the same time, there are distinctive differences in biochemical pathways, particularly in maintenance processes such as ion transport and protein turnover, which could drive differences between ECE and NUE. There was a strong

correlation between NUE and ECE at all stages of this study which suggests that the common pathways and processes for ECE and NUE were dominant.

3.4.2 Contribution of body reserves to the relationship between ECE and NUE

There is a large body of literature about the contribution of body reserves to synthesis of milk constituents, which can be as large as 30% of requirements (Bauman and Currie, 1980; Loor et al., 2006; Rocco and McNamara, 2013), thus having a large effect on FCE over short periods of time. The largest difference between NUE and ECE occurred immediately after calving, particularly for the older animals that received the higher concentrate allocation in the first lactation. The higher coefficient of variation for ECE estimates in this study reflects the more variable contribution of body reserves to milk energy in comparison to milk protein, particularly in the early part of the second lactation when mobilization of body reserves was most variable (Dewhurst et al., 2002).

BCS change and N balance data were presented previously (Dewhurst et al., 2002; Moorby et al., 2002), however it is difficult to use them to quantify the relative contribution of body fat and protein to milk components. Schroder and Staufenbiel (2006) estimated that for each unit of change, fat and protein contribute 93 and 7% respectively to total tissue loss or gain and it has been calculated that 1kg of tissue represents 0.64kg fat, 0.28kg water and only 0.08kg of protein (Madhav et al., 1996). Immediately post partum, it has been estimated that dairy cows can utilise up to 1000g/d of protein to sustain the mammary gland (Bell et al., 2000) and N balance measurements have reported up to 21kg of protein is mobilised in early lactation (18% CP diet) (Botts et al., 1979). In contrast, mobilization of body fat is much higher than protein during early lactation (Smith and McNamara, 1990; Sumner-Thomson et al., 2011). These differences reflect the fact that genetic regulation of muscle mass (Lee and McPherron, 1999; Bell et al., 2000) operates independently of genetic variation in body fat (Cases et al., 1998; Schennink et al., 2007; Sumner and McNamara, 2007; Prokesch et al., 2009; Thering et al., 2009; Sumner-Thomson et al., 2011).

Whilst the above analysis looks at variation within the lactation cycle, it is interesting to look at the complete lactation cycle. Variation in LWT change over the full lactation cycle represented only a very small proportion of ME intake and explained less than 10% of variation in the relationship between ECE and NUE.

3.4.3 Relationship between production level and feed efficiency

The higher FCE in early lactation (Figure 3.1) is the combined effect of the utilisation of body reserves and the spreading of maintenance costs across more milk production (Vandehaar and St-Pierre, 2006). As feed intake and production level increase, the proportion of feed nutrients used for maintenance purposes becomes smaller. This process is common to both NUE and ECE, but there are relative differences in maintenance costs for energy and protein (ARC, 1980; AFRC, 1992). Comparing predicted NUE (using equation 1) and ECE (using equation 2) for the maximum and half maximum yields of milk N and milk energy respectively supports this effect. Predicted NUE for 50 g milk N/d was 65.4% of that predicted for 100 g milk N/d, whilst predicted ECE when producing 42.5 MJ milk energy/d was 60.1% of that for 85 MJ milk energy/d. Calculations using the requirements for maintenance and milk production in the UK MP system (ARFC, 1992) and Metabolisable energy system (ARC, 1980) showed the same relative difference. However, the calculated effects were smaller than predicted by the AFRC and ARC equations; NUE at 50g MP (g/d) was 80.6% (0.61 g/g) of NUE at 100g MP (g/d) (0.76 g/g), whilst ECE at 42.5 (MJ/d) energy was 75.5% (0.51 MJ/MJ) of ECE fed at 85 (MJ/d) energy (0.68 MJ/MJ). This is partly because the calculations did not include the energy and protein requirements for pregnancy, nor efficiency losses associated with gaining and losing body reserves over the lactation cycle.

3.4.4 Common biochemical pathways for ECE and NUE

Differences in milk yield would result in ‘dilution of maintenance’ effects on both ECE and NUE and are major contributors, along with the high efficiency of nutrient use for milk synthesis (Baldwin, 1968), to the strong relationship between the two

(Figure 3.2). The mammary gland has a high efficiency for the capture of amino acids for milk synthesis, however the efficiency of utilisation can be variable depending on the type and supply of amino acids (Lapierre et al., 2005). Synthesis of non-essential amino acids and gluconeogenesis from amino acids are examples of the inter-changes between energy and protein metabolism that are subject to the concerted regulation. The strong relationship between ECE and NUE when driven by animal effects, and particularly outside the early lactation period, suggests that effects due to differences in biochemical pathways associated with ECE and NUE are relatively small. These differences are visible in the variability in Figure 3.1 which are probably due to between-animal differences in the use of ATP and amino acids in maintenance processes such as ion transport and protein turnover.

3.5 Conclusions

There was a strong correlation between NUE and ECE as estimates of FCE. This appears to be driven by commonality in the effects of dilution of maintenance costs for energy and protein, as well as pathways for synthesis of milk components. Mobilization of body reserves introduced variation into the relationship between ECE and NUE, particularly in early lactation, and makes NUE a more stable target than ECE. For the whole lactation cycle, there were small differences in the relationship between ECE and NUE that are driven by differences in metabolic efficiency of maintenance processes. Given these results, this makes NUE a reliable measurement to use to evaluate the relationship between NUE and N isotope fractionation in further studies.

CHAPTER 4

Plasma nitrogen isotopic fractionation and feed efficiency in growing beef heifers

4.1 Introduction

In this chapter the relationship between N isotopic fractionation was investigated as a phenotypic marker for feed efficiency in beef animals and for its potential to provide a rapid low cost estimate of feed efficiency in large groups of cattle. Improvements in feed efficiency are essential for the beef farmer because of increasing costs of production; feed is the largest variable cost and an important determinant of profitability in beef production (Ashfield et al., 2013). Identification of, and genetic selection for, feed efficient cattle is an important approach to achieve these multiple objectives (Herd et al., 2004).

Feed conversion ratio, defined as the ratio of feed intake to LWT gain (Archer et al., 1999) and FCE defined as the units of output per unit of feed (Crews jr, 2005) are traditional measures of feed efficiency commonly used in assessing beef cattle. Residual feed intake, defined as the difference between an animal's measured feed intake and expected intake based on its weight and average daily gain, is an alternative method of measuring feed efficiency in beef cattle, and is independent of growth rate and LWT (Koch et al., 1963).

Incorporation of feed efficiency measurements into cattle breeding strategies has been slow (Herd et al., 2004). Feed efficiency is costly and laborious to measure, even with automated systems. It is necessary to record feed intake and growth over at least 70 days in order to have accurate estimates of efficiency (e.g. Herd et al., 2003) and it is not possible to easily apply this approach to grazing animals (Archer et al., 1997). There is also uncertainty about the composition of body reserves and live-weight change, as well as nutrient requirements for pregnancy which make these measurements even more difficult, especially with pregnant and (or) lactating animals (Dewhurst et al., 2000).

More recently, there has been an increased interest in longer-term strategies of genetic and genomic selection for feed efficiency or its components such as digestive efficiency, metabolic efficiency or animal activity (Moore et al., 2009). Success in identifying genetic and genomic associations with complex traits such as feed efficiency depends on being able to measure the phenotype in large numbers of animals representing a broad cross-section of genetic variation (Robinson et al., 2004). A number of studies have sought biochemical markers for feed efficiency (measured as RFI; Richardson et al., 2004; Kelly et al., 2010; Lawrence et al., 2012) in response to these difficulties. Unfortunately, these markers have only been poorly correlated with feed efficiency measurements. These relationships may have been influenced by physiological factors including dietary intake, digestibility, metabolism of nutrients, physical activity and thermoregulation (Herd et al., 2009), and external factors such as environment and health (Arthur et al., 2004). Identification of phenotypic markers can also be used to aid in constructing diets to match the energy requirements of cattle and compare the efficiency of nutrient use for different feeds.

We have investigated an alternative marker for feed efficiency based on the phenomenon of N isotopic fractionation (Macko et al., 1986) that occurs during a number of metabolic pathways. Nitrogen isotopic fractionation occurs when the incorporation of natural isotopes ^{14}N and ^{15}N into products is slightly different to their ratio in precursors because of differences in the mass of small compounds containing either ^{14}N or ^{15}N. As a result of N isotopic fractionation, urine is depleted in ^{15}N relative to the diet, whilst animal proteins are enriched (Minagawa and Wada, 1984; Sutoh et al., 1987; Poupin et al., 2011). In particular, enrichment of tissue protein occurs during transamination and deamination reactions in the conversion of excess dietary protein into urinary urea (Balter et al., 2006), protein turnover and recycling (Balter et al., 2006; Poupin et al., 2011; Martinez del Rio and Carleton, 2012) and rumen metabolism (Wattiaux and Reed, 1995). The magnitude of fractionation depends on the efficiency of assimilation (Martinez del Rio and Carleton, 2012) and metabolic rate (Smith et al., 2010) and varies between tissue type and metabolic pools (De Niro and Epstein, 1981; Caut et al., 2009). N fractionation

has been a useful predictor of N partitioning in non-ruminants e.g. measurements in plasma protein from rats (Sick et al., 1997) and hair protein from horses, cattle and goats (Sponheimer et al., 2003). There have also been previous reports of the use of N isotopes to investigate the effects of changing dietary protein in beef cattle (Sponheimer et al., 2003), the mechanisms of fractionation in ruminal microbes (Wattiaux and Reed, 1995) and for predicting NUE in dairy cattle (Cheng et al., 2011).

Nitrogen use efficiency, defined as the efficiency of converting feed N into animal protein N, is an important component of overall feed efficiency (Wheadon et al., 2012a), as muscle protein is the most valuable component of the carcass in beef cattle. Since body composition was not measured in the present study, we have related N isotopic fractionation to overall FCE. We expect a strong relationship between NUE and FCE, particularly when offering the same diet. This is because NUE is a component of overall FCE, and dilution of maintenance effects, as well as a number of important cell and organ maintenance costs affect both NUE and FCE (Herd et al., 2004). The adjustments involved in calculating RFI and other more complex measures of feed efficiency make them less likely to be related to N isotopic fractionation than simple measures of input/output such as FCE investigated in this work.

The objective of this chapter was to investigate the relationship between N isotopic fractionation, measured in plasma, and estimates of FCE in growing beef heifers. We also investigated the repeatability of N isotopic fractionation over a 3-month period and we hypothesized that there would be no change in the relationship between N isotope fractionation and FCE over time.

4.2 Materials and methods

4.2.1 Animals and their management

All animal procedures performed in this study were conducted under experimental licence from the Irish Department of Health and Children in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendment of Cruelty to Animals Act 1876) Regulation 2002 and 2005. This study used plasma samples from eighty-four beef heifers (62 Simmental and 22 Simmental × Holstein-Friesian) sampled on one occasion towards the end of a period of recording FCE. A subset of 20 heifers from the same population (15 Simmental and 5 Simmental × Holstein-Friesian) was sampled over 4 time points spaced across the FCE recording period. Further details of the original study have been described by Lawrence et al. (2012). The heifers were on average 299 (s.d. = 48.3) days old and weighed 311 (s.d. = 48.8) kg at the start of the experiment. They were housed in pens of 4 to 6 animals (lying area 2.87 m^2/animal) in a shed with slatted floors and adapted to their diet for 3 weeks prior to a 12-week recording period. Heifers were individually offered grass silage *ad libitum* and were fed 2 kg/day concentrates (at 0800h) through Calan gates (American Calan Inc., Northwood, U.S.A). Daily feed intakes were recorded and animals were weighed at 3-week intervals.

4.2.2 Sample collection and analysis

Blood samples were obtained by jugular venepuncture from all animals on day 79, and from the subset of 20 animals on days 16, 37, 58 and 79 of the experimental period. The former samples were collected into 4 ml evacuated tubes containing sodium citrate (Greiner Vacuette, Cruinn Diagnostics, Dublin, Ireland), whilst samples from the subset of 20 animals were collected separately into 9 ml evacuated tubes containing lithium heparin (Greiner Vacuette, Cruinn Diagnostics, Dublin, Ireland). Blood samples were then centrifuged (2500 g, 20 minutes, 4°C), and the plasma stored at –20°C until analysis.

Grass silage and concentrate offered was sampled three and two times weekly and, composited weekly and fortnightly, respectively. These were analysed according to the methods described by Owens et al (2008) with the exception that the DM content of the grass silage which was determined by drying a sub-sample at 98˚C for 48 hours. Samples of the concentrates and grass silage (n=20; all in duplicate), as well as plasma samples, measured in whole plasma (n=164 plus 10% duplicates) were analysed for ^{15}N content by isotope-ratio mass spectrometry (Iso-Analytical Ltd., Crewe, UK). Nitrogen-15 results are expressed in delta units relative to standard air ($\delta^{15}N$, ‰). N isotopic fractionation, the difference between $\delta^{15}N$ for plasma and $\delta^{15}N$ for the diet is termed $\Delta\delta^{15}N$, or simply $\Delta^{15}N$. Average diet $\delta^{15}N$ was calculated for each animal by weighting $\delta^{15}N$ for silage and $\delta^{15}N$ for concentrate based on N intake (g/d) of each diet component.

4.2.3 Statistical analysis

Residual feed intake was calculated as the difference between actual dry matter intake (DMI) and expected DMI, using regression models to predict DMI (Lawrence et al., 2012). For RFI, expected DMI was computed for each animal using a multiple regression model, regressing DMI and mid-test live weight and average dairy gain with breed included as a fixed effect. The coefficient of determination from this regression model was equal to 0.66 ($P<0.001$) and the model was subsequently used to predict DMI for each animal. Actual DMI was calculated as the mean of the daily quantities of feed offered minus the subsequent refusals over the 84-day recording period, corrected for DM concentration.

The relationship between plasma $\delta^{15}N$, $\Delta^{15}N$, FCE, RFI and $W^{0.75}$ were described using linear regression (Genstat release 10.1; Lawes Agricultural Trust (Rothamsted Experimental Station), 2007) using breed as a factor in the model. ANOVA was used to establish the effect of breed and sire on N isotope fractionation and feed efficiency. Pair-wise Pearson correlations were used to show the relationship of N isotopic fractionation between time points and a REML repeated measures model was used to analyse changes in the relationship between FCE and N isotope

fractionation over time. Plasma $\delta^{15}N$ was used as the response variate and NUE as the fitted model, plus random factors metabolic live weight and forage to concentrate ratio. The goodness of fit of the variance-covariance structure used for fitting the data was a uniform covariance structure (compound symmetry) matrix.

4.3 Results

Eighty-six heifers commenced the experiment, but values were excluded from one sick animal and one extreme outlier (confirmed using Cook's test); these exclusions had no effect on the relationships identified. The average chemical composition of the grass silage and concentrates across the experiment is described in Table 4.1. Across the whole population, average DM intake (g/g) was 5.82 (s.d. = 0.74), silage DM intake averaged 4.1 (s.d. = 0.74) kg/day and concentrate intake was 1.72 kg/day, so that the forage proportion of total DM intake (g/g) averaged 0.70 (s.d. = 0.038). Mean mid-test LWT was 333 (s.d. = 47.6) kg, average daily gain was 0.53 (s.d. = 0.183) kg, FCE (g live-weight gain/ g DM intake) was 0.09 (s.d. = 0.028) and average RFI was 0 (s.d. = 0.428). For the subset of 20 heifers average DM intake (kg/day) was 5.82 (s.d. = 0.59), silage DM intake averaged 4.1 (s.d. = 0.59) and concentrate was 1.72 kg/day, so forage proportion of total DM intake (g/g) averaged 0.70 (s.d. = 0.031). Mean mid-test LWT was 339 (s.d. = 53.7) kg, average daily gain was 0.55 (s.d. = 0.164) kg, average FCE was 0.10 (s.d. = 0.030) and RFI was -0.079 (s.d. = 0.472).

Table 4.1 Average chemical composition (s.d.) of feed samples collected over the experimental period.

	Diet component	
	Grass silage	Concentrate
DM (g/kg)	244.3	85.79
pH	3.9	ND
Composition of DM (g/kg DM unless otherwise stated)		
in-vitro DMD*	744	858
in-vitro DOMD†	688	ND
OMD‡	762	ND
Ash	98	85
CP	136	140
NDF	511	215
Starch	ND	269
Fermentation characteristics (g/kg DM)		
Lactic acid	43	ND
Acetic acid	80	ND
Propionic acid	4.9	ND
Butyric acid	12.9	ND
Ethanol	57.4	ND
Ammonia N (g/kg total N)	73	ND
$\delta^{15}N$ (‰)	5.74	3.20

ND, not determined.
*Dry matter digestibility, measured *in vitro.*
†Digestible organic matter in the total DM, measured *in vitro*.
‡Organic matter digestibility, measured *in vitro*.

Although feed samples were drawn from the same batches, there was slight variation in $\delta^{15}N$ values, so average values were used in calculating $\Delta^{15}N$. Average $\delta^{15}N$ values of the concentrates and grass silage were 3.20 ‰ (s.d. = 0.189) and 5.74 ‰ (s.d. = 0.103) and the weighted value for the whole diet were 4.96 ‰ (s.d. = 0.082) for the subset of 20 animals and 4.95 ‰ (s.d. = 0.098) for all 84 animals. Plasma $\delta^{15}N$ for each of the four time points averaged 8.85 (s.d. = 0.43), 8.80 (s.d. = 0.40), 8.84 (s.d. = 0.41) and 8.69 (s.d. = 0.48), respectively, ranging from a 3.04 to 5.11 unit enrichment relative to the diet. The average $\delta^{15}N$ of plasma for all 84 animals at day 79 was 8.53 (s.d. = 0.364) ‰, ranging from a 3.51 to 5.14 unit enrichment relative to the diet.

Although there was a small range in overall diet $\delta^{15}N$ (a consequence of the differences in forage/concentrate ratio consumed), there was no significant relationship between diet $\delta^{15}N$ and plasma $\delta^{15}N$ for the whole population or any of the repeated time points for the subset of heifers. Linear regression analysis using data from all animals at day 79, as well as for the subset of 20 animals at each of the 4 time points, showed a significant negative relationship between plasma $\delta^{15}N$ and FCE in each case (Table 4.2).

Table 4.2 Coefficients of determination (r^2) or coefficients of multiple determination (R^2) for the relationships between feed conversion efficiency (FCE; g LWT gain/ kg DM intake) and plasma $\delta^{15}N$, $\Delta^{15}N$ (plasma $\delta^{15}N$ – diet $\delta^{15}N$) and mid-test $W^{0.75}$, with average s.e. All relationships significant at $P<0.001$ level.

	Day of Experiment					
	16	37	58	79 (n=20)	79 (n=84)	s.e.m.
Plasma $\delta^{15}N$	0.52	0.63	0.69	0.59	0.28	0.284
$\Delta^{15}N$	0.47	0.56	0.64	0.56	0.35	0.284
Plasma $\delta^{15}N$ + Mid test $W^{0.75}$	0.81	0.83	0.84	0.74	0.44	0.22
$\Delta^{15}N$ + Mid test $W^{0.75}$	0.72	0.71	0.75	0.68	0.41	0.244

Nitrogen isotopic fractionation ($\Delta^{15}N$; plasma $\delta^{15}N$ – diet $\delta^{15}N$) was also negatively related to FCE for all sample time points (Figure 4.1 (a)–(e), equations (1)-(5)); the subset showed statistically stronger relationships (higher r^2 and R^2) than were observed for the whole population.

Day 79 (n=84): $\Delta^{15}N = 4.216$ (s.e. = 0.101) ─ 7.36 (s.e. = 1.10) × FCE (g/g) (1)

Day 16 (n=20): $\Delta^{15}N = 4.781$ (s.e. = 0.231) ─ 9.33 (s.e. = 2.32) × FCE (g/g) (2)

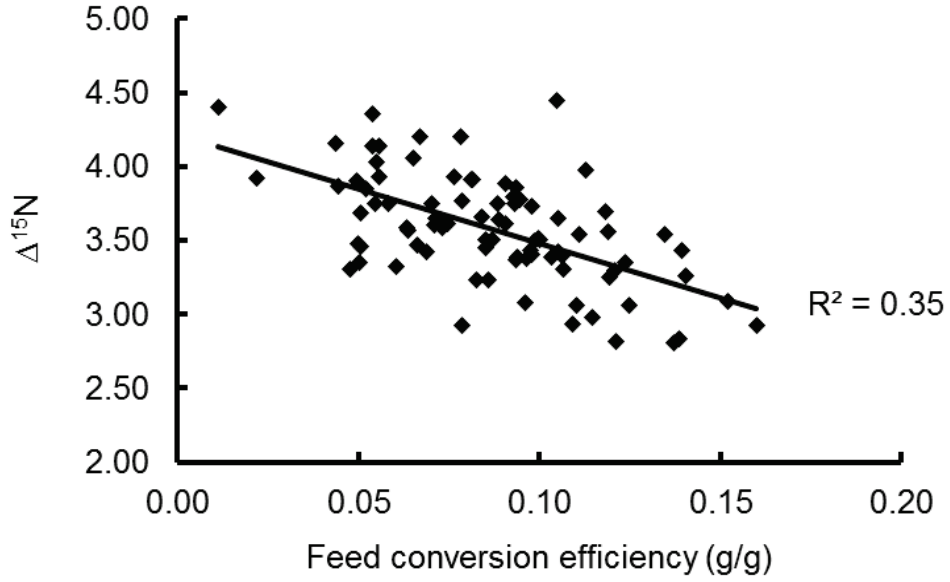
Day 37 (n=20): $\Delta^{15}N = 4.743$ (s.e. = 0.197) ─ 9.49 (s.e. = 1.98) × FCE (g/g) (3)

Day 58 (n=20): $\Delta^{15}N = 4.854$ (s.e. = 0.179) ─ 10.25 (s.e. = 1.80) × FCE (g/g) (4)

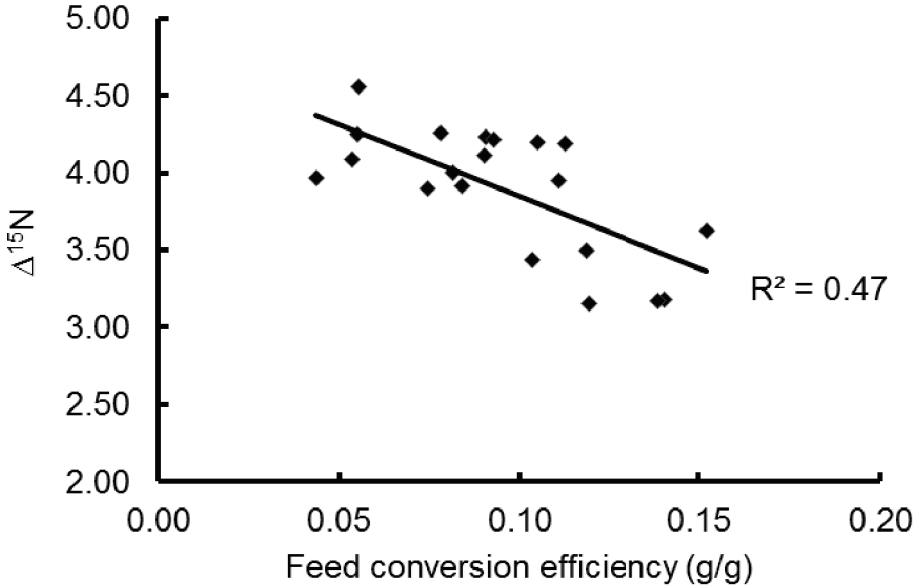
Day 79 (n=20): $\Delta^{15}N = 4.789$ (s.e. = 0.232) ─ 11.11 (s.e. = 2.33) × FCE (g/g) (5)

There was no significant difference between the slopes on different days (P = 0.848). Plasma $\delta^{15}N$ measurements from the same animals in the subset were significantly correlated over adjacent time points (P<0.001 correlation between all days) (average $r = 0.96$), and the degree of correlation for individuals decreased with greater time separation between samplings (e.g. $r = 0.84$ between the 20 animals on the first and last sampling dates).

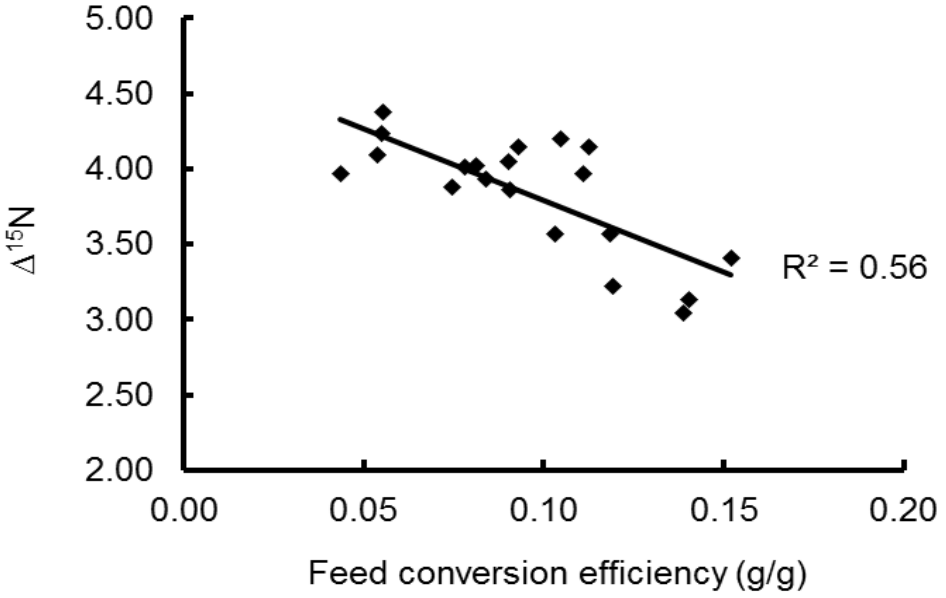
(a) Day 79 (n=84)



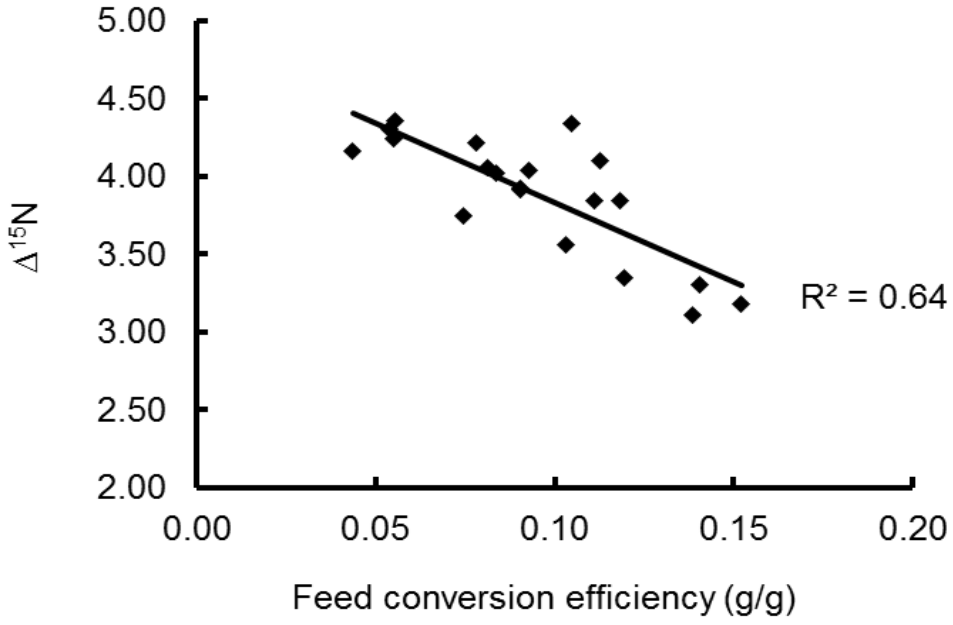
(b) Day 16 (n=20)



(c) Day 37 (n=20)



(d) Day 58 (n=20)



(e) Day 79 (n=20)

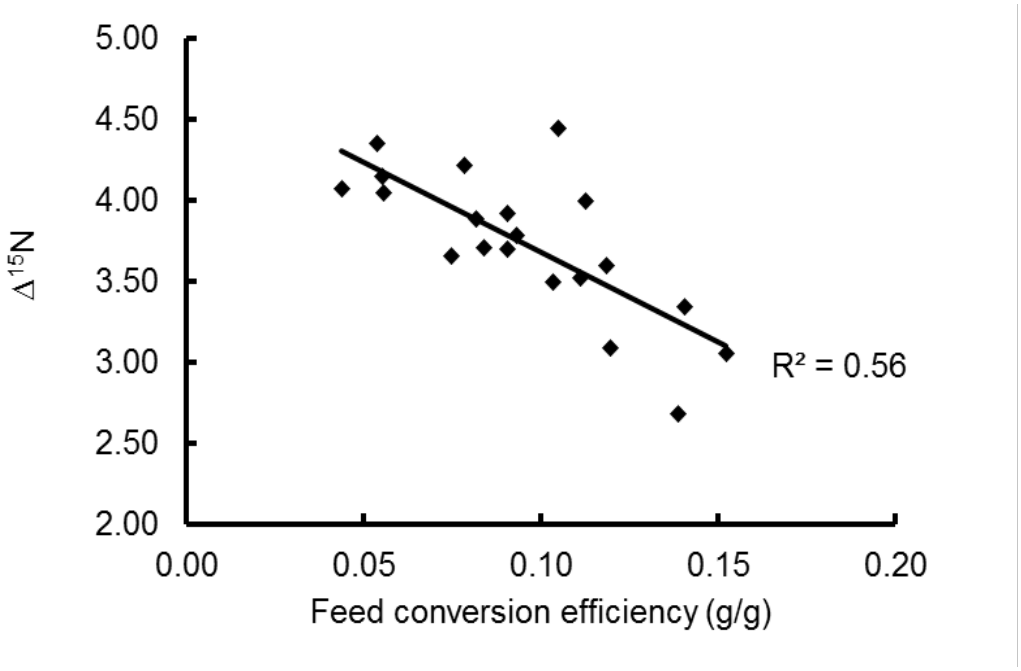


Figure 4.1 Relationship between N isotopic fractionation ($\Delta^{15}N$; the difference between plasma $\delta^{15}N$ and diet $\delta^{15}N$) and FCE (g of LWT gain per g of DMI) for (a) all animals at day 79 and (b-e) a subset of 20 animals at days 16, 37, 58 and 79.

There was no significant relationship between FCE and DMI (P = 0.16; r^2 = 0.06). There was a weak (r^2 = 0.10), but significant (P = 0.002) negative relationship between FCE and mid-test metabolic weight ($W^{0.75}$). Nonetheless, adding $W^{0.75}$ in a multiple regression analyses explained an additional and highly significant part of the variation in plasma $\delta^{15}N$ and $\Delta^{15}N$ (plasma $\delta^{15}N$ – diet $\delta^{15}N$) across all time points (Table 4.2). There was no significant relationship between plasma $\delta^{15}N$ and RFI (P = 0.21; r^2=0.07), nor between $\Delta^{15}N$ and RFI (P = 0.82: r^2=0).

There was a significant difference in FCE between breeds in this study (P<0.001). Pure Simmental heifers had lower FCE (0.076; s.d. = 0.003) than Holstein-Friesian × Simmental heifers (0.119; s.d. = 0.004). Simmental heifers also had significantly higher plasma $\delta^{15}N$ (P<0.001) and $\Delta^{15}N$ (P<0.001) than Holstein Friesian × Simmental cross heifers; 8.63 (s.d. = 0.04) and 8.22 (s.d. = 0.06) and, 4.38 (s.d. = 0.04) and 3.92 (s.d. = 0.06), respectively. Despite significant differences in FCE and N isotopes between breeds, there was no significant effect on the relationship between FCE and plasma $\delta^{15}N$ or $\Delta^{15}N$. FCE (g/g) also differed across sire groups (P<0.001), ranging from 0.06 (s.d. = 0.010) to 0.13 (s.d. = 0.001). There were 15 sires for the larger group, and three sires for the subset group. Plasma $\delta^{15}N$, and as a result plasma $\delta^{15}N$ – diet $\delta^{15}N$ were significantly different between sire groups (P<0.001 and P<0.001). Plasma $\delta^{15}N$ and $\Delta^{15}N$ ranged from 8.17 (s.d. = 0.12) to 8.97 (s.d. = 0.11) and 3.81 (s.d. = 0.11) to 4.69 (s.d. = 0.10) for different sire groups, respectively. Plasma $\delta^{15}N$ and $\Delta^{15}N$ tended to be higher in sire groups with lower FCE. FCE and average daily gain was slightly higher for the subset of 20 heifers compared to the whole population (P<0.001 and P<0.001, respectively).

4.4 Discussion

NUE in cattle can be improved by increasing N retention in muscle at a similar intake, by reducing N intake at a similar N retention, or by a combination of both. Live weight gain is the main driver for N retention in growing animals and as muscle protein is the most valuable component of the carcass in beef cattle it is essential to identify animals with higher NUE which will aid in lowering the impact of deterimental N emissions to the environment (Gill et al., 2010). Phenotypic markers such as N isotopic fractionation used in this study have potential to be used as a proxy to estimate feed efficiency in cattle where diet composition or intake cannot be recorded. This will accelerate collection of feed efficiency data for large numbers of animals in breeding programmes.

4.4.1 Comparison of N isotopic fractionation with earlier studies

Nitrogen isotopic fractionation ($\Delta^{15}N$) between whole plasma and the diet overall averaged 3.74 in this study (range 3.04 to 5.11 ‰), which is lower than the range measured previously with growing or mature ruminants (3.80 to 6.94‰ for treatment means; Table 4.3). The plasma urea fraction is generally depleted in $\delta^{15}N$ compared to both the diet and the plasma protein fraction (Poupin et al., 2011). Measuring $\Delta^{15}N$ in different fractions within plasma (e.g. protein and urea) could affect results, but effects in this study would be negligible since urea N made up less than 1% of plasma N. For the same reason, we calculate that the fact that the highest $\Delta^{15}N$ in the literature (Table 4.3) was for plasma protein is purely coincidental. There was only a weak relationship (r^2 = 0.1) between plasma urea N and FCE in this study, which taken together with the low level of urea-N relative to protein-N in plasma suggests that the urea fraction of plasma would not affect the relationship between FCE and plasma $\delta^{15}N$.

Table 4.3 Literature values for N isotopic fractionation in ruminants ($\Delta^{15}N$; $\delta^{15}N$ in blood minus $\delta^{15}N$ in the diet)

Reference	Physiological state	Animal description	Diet	$\Delta^{15}N$	Sample type
Present study*	Growing	Growing heifers	Grass silage/concentrates	3.58	plasma
Present study†	Growing	Growing heifers	Grass silage/concentrates	3.90	plasma
Steele and Daniel, 1978	Growing/mature	Angus steers	Ryegrass/white clover silage	4.2	blood
Sponheimer et al. 2003, ‡	Growing/mature	Cattle	Lucerne hay	4	blood
Koyama et al. 1985	Growing/mature	Beef cattle	Rice straw	3.8	blood
Sponheimer et al. 2003, ‡	Growing/mature	Goats	Lucerne hay	4.7	plasma
Sutoh et al. 1989	Mature	Goats, 2 year old females	Lucerne hay cubes	4.8	plasma
Sutoh et al. 1993	Mature	Wethers, 4 years old	Lucerne hay cubes	6.94	plasma protein
Darr and Hewitt, 2008	Mature	Deer, 2 year old males	Lucerne	5.67	serum
Darr and Hewitt, 2008	Mature	Deer, 2 year old males	Lucerne/maize (3:2)	6.2	serum
Koyama et al. 1985	Lactating	Beef cows, milking	Pasture	2.59	blood
Sutoh et al. 1993	Lactating	Lactating dairy cows	Forage/concentrates (1:1)	2.37	plasma
Cheng et al. 2013	Lactating	Lactating dairy cows	Grass	3.19	plasma
Jenkins et al. 2001, ‡	Lactating	Sheep, early lactation	Hay	5.1	plasma
Jenkins et al. 2001, ‡	Lactating	Sheep, early lactation		4.5	plasma
Jenkins et al. 2001	Neonate	Deer calves: 12-14 days old	Milk	1.9	plasma
Jenkins et al. 2001	Neonate	Lambs: 12-14 days old	Milk	3	plasma

* whole population (n=84), †subset heifers averaged over time (n=20)

‡ also unpublished data cited by Robbins et al. 2005

Whilst most previous studies provided only limited descriptions of the animals, many involved mature animals that would have ceased growing and so use dietary N relatively inefficiently, resulting in a high level of isotopic fractionation ($\Delta^{15}N$). Lower $\Delta^{15}N$ were observed in studies with suckling young and in most, but not all, studies with lactating ruminants. It would be expected that these more productive animals would be using N more efficiently, partitioning a higher proportion of N to muscle or milk protein and less to urea.

4.4.2 Repeatability of N isotopic fractionation measurements

In this study we demonstrated a consistent and repeatable strong relationship between N isotopic fractionation and FCE which will be useful for application of the approach in animal breeding. The correlation between plasma $\delta^{15}N$ at the different time points was also usefully strong. It appears that FCE remained constant over the measurement period and $\Delta^{15}N$ values remained stable despite the natural variation in growth rate that may have been increased because of heifers entering puberty at different stages during the experiment (Ciccioli et al., 2005).

4.4.3 Relationship of N isotopic fractionation with feed conversion efficiency

Nitrogen isotopic fractionation ($\Delta^{15}N$) was a good indicator of FCE in this study, and it seems likely that this relationship was driven by the partitioning of N between liveweight gain and excretion in urine. The negative relationship between $\Delta^{15}N$ and FCE is consistent with the increased $\Delta^{15}N$ when cattle and goats were fed diets containing higher protein levels (Sponheimer et al., 2003). The relationship between $\Delta^{15}N$ (plasma $\delta^{15}N$ – diet $\delta^{15}N$) and FCE was probably stronger for the subset of 20 animals because they were more homogenous (lower s.d. for LWT) and genetically more similar. Plasma $\delta^{15}N$ differed between breed and sire groups, providing preliminary evidence that it will be possible to use this approach to select for beef cattle with higher FCE. N isotope fractionation only explained 1% of the variance of RFI; as expected, N isotopic fractionation was more related to simple measures of input and output (i.e. FCE) than complex measures of efficiency such as RFI.

Since the composition of LWT gain was not measured in this study, it is likely that some of the unexplained variation in the relationships between plasma $\delta^{15}N$ or $\Delta^{15}N$ (i.e. plasma $\delta^{15}N$ – diet $\delta^{15}N$) and FCE results from variation in the relationship between NUE and FCE. The inclusion of $W^{0.75}$ accounted for some of the variation in the relationship between $\Delta^{15}N$ and FCE (Table 4.2) because the N content of live-weight gain declines as animals grow (ARC, 1980). Average values for the protein content of live-weight gain are 6.3% lower for 450 kg cattle in comparison with 250 kg cattle (ARC, 1980), implying a reduction in NUE at the same FCE. Fractionation of N isotopes in the rumen could explain additional variation (Steele and Daniel, 1978; Wattiaux and Reed, 1995), though the strong relationships with FCE and use of a consistent diet suggest that any effect would have been small.

N isotopic fractionation ($\Delta^{15}N$) explained more variation (based on regression models) in FCE in animals fed the same diet than single blood metabolites or hormones that were used to predict RFI in earlier studies. In these animals, Lawrence et al. (2012) found no significant relationships between blood metabolites and RFI, except for creatinine. Kelly et al. (2010) reported weak relationships between feed conversion ratio (FCR), defined as the ratio of feed intake to live weight gain, and plasma leptin (r^2 = 0.23), urea (r^2 = 0.18) and NEFA (r^2 = 0.10) in heifers and Richardson et al. (2004) found only weak relationships between FCR and plasma glucose (r^2 = 0.21), aspartate aminotransferase (r^2 = 0.20) and albumin (r^2 = 0.23) in steers.

The partitioning of N between protein (muscle tissue or milk) and urea is affected by protein supply and protein quality (Sick et al., 1997; Poupin et al., 2011) and both aspects have been used to explain differences in N isotopic fractionation. The first effect suggests that as dietary protein supply increases, an increasing proportion of N is directed to urine, so that fractionation increases. The second effect emphasises increased fractionation as a result of decreased protein quality and consequent increased losses as urine N. In this study, animals received the same diet, so effects must be mainly due to between-animal variation in feed utilisation and the variation in silage quality was small, so the between-animal variation in N partitioning

depends on the ability of individual animals to utilise protein, which will depend on their genetic make up and management history. Nitrogen fractionation in this study was able to detect the animal variation associated with N partitioning in FCE.

4.5 Conclusion

We have demonstrated a highly significant negative relationship between N isotopic fractionation between plasma and the diet ($\Delta^{15}N$) and feed efficiency in growing beef heifers. The relationship between N isotopic fractionation and FCE remained constant and the rate of fractionation changed slowly over time, which would be useful for application in cattle breeding. N isotope fractionation may be useful in evaluating feed efficiency without measuring feed intake, and even diet composition, and be used to compare the nutrient use efficiency of different feeds.

CHAPTER 5

The relationship between nitrogen isotope fractionation and nitrogen-use efficiency in dairy cattle on pasture

5.1 Introduction

Chapter 4 demonstrated a highly significant negative relationship between N isotopic fractionation (between the diet and plasma; $\Delta^{15}N$) and feed efficiency in growing beef heifers. N isotopic fractionation may be a useful tool in cattle breeding decisions, and may also be used to compare nutrient use of different feeds. Phenotypic markers for feed efficiency would also be particularly useful in free grazing animals (Prendiville et al., 2009) without measuring feed intake, and even diet composition, providing that animals are fed with identical diets. This is a challenging objective with grazing cows as there are variations in diet composition even within the same location and there are also behavioural differences e.g. satiety and sward selectivity, therefore animals do not necessarily receive identical diets. With similar environmental effects on feed efficiency occurring within a study, the differences in N fractionation between animals receiving similar diets may be due to behavioural differences in intake, variations in pasture composition between paddocks, but also due to between-animal variation in feed utilisation as a result of genetic effects, which will ultimately control the partitioning of nutrients and resources for metabolic activities (Gibson, 1986).

Nitrogen isotopic fractionation explained more variation in FCE for animals on the same diet (based on the variance explained by regression models) in chapter 4 than single blood metabolites or hormones that were used to predict RFI in earlier studies (e.g. Richardson et al., 2004; Kelly et al., 2010; Lawrence et al., 2012). Since N isotope fractionation was related to FCE indirectly (and NUE is an important component of FCE), we would expect it to be related to NUE directly, as it measures the partitioning of N to milk or muscle and urine respectively. There are important differences between growing beef and dairy cattle in respect to feed efficiency

(Castillo et al., 2000; Arthur et al., 2004; Berry and Crowley, 2013). Efficiency for milk synthesis is high for dairy cows (83%; Baldwin, 1968; 81-84%; Onken et al., 2011) and the efficiency of amino acid utilisation is higher for lactating animals than growing animals (AFRC, 1992). Therefore in the study reported in this chapter, further investigation was made of the use of N isotope fractionation as a marker for NUE in free grazing lactating dairy cows.

5.2 Materials and methods

5.2.1 Experimental design

Samples were collected at the 67 hectare Ballydague research farm (Teagasc, Cork, Republic of Ireland; Latitude 50°07'N, Longitude 08°16'W), which has 180 milking cows in a 300-day grazing season. The overall aim of the study was to provide comparative animal performance between Holstein-Friesian, Jersey and Holstein-Friesian × Jersey cows, evaluated across varying stocking intensities, to ascertain differences in animal performance/profitability that may occur when stocking rate is increased (genotype × environment interactions).

Samples were collected from 135 cows that were managed in 3 breed groups (Holstein-Friesian, Jersey and Holstein-Friesian × Jersey). Forty-five cows of each breed were randomly assigned to one of three stocking rate (SR) treatments in spring 2009 (high; 3 cows/ha, medium; 2.75 cows/ha and low; 2.5 cows/ha) (15 cows per treatment) which were blocked on breed, parity (1-4), calving date and milk yield/milk solid yield, as described by Thackaberry et al. (2011). Each of the 9 treatment groups were allocated to a fixed farmlet and each group had 20 paddocks that were grazed in a rotational system. High, medium and low SR groups were able to graze to a target post-grazing sward height of 3.3cm, 4.2cm and 4.9 cm respectively (measured by Rising Plate Meter; Jenquip, Feilding, New Zealand) before being moved, restricting land space and grazing material in the high SR groups, therefore optimizing efficiency.

5.2.2 Sampling

The overall study was conducted over two complete lactations over 2 years. Milk yield was recorded daily throughout the study using electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). Milk composition was determined weekly from successive am and pm milk samples (Milkoscan 203, Foss electric, DK-3400 Hillerod, Denmark). Live weight was recorded weekly using a calibrated weighing scale (Dairymaster, Causeway, Co. Kerry, Ireland). BCS was assessed every 4 weeks using a 5 point scale (Lowman et al., 1976).

The measurements reported in this chapter were made during the first year of the study; the mean calving date was 14th February 2009 and average days in milk were 151 (s.d. = 24) and 182 (s.d. = 24) for periods 1 and 2. Blood samples were collected from 2 periods in mid-lactation (July and August). Plasma was prepared by centrifuging at 3000g for 15 minutes at 4^{o}C and stored at -20 prior to analysis. Pasture samples were taken from each of the 9 paddocks on the same dates as blood samples. Sub-samples of herbage were collected, weighed and dried overnight at 60 °C for DM content. Dry matter digestibility (DMD), ash and water soluble carbohydrates were determined by NIRS (5000/6500, FOSS Electric, Denmark). NIRS calibration was maintained by reference to wet chemistry methods. The CP concentration of the samples was analyzed using a Leco N analyzer (Leco FP-528; Leco Corporation, St., Joseph, MI, USA).

Sub-samples of pasture and plasma were analysed for the ratio of ^{14}N to ^{15}N, measured as $\delta^{15}N$ (‰) by isotope ratio mass spectrometry (IRMS; Iso-Analytical, Crewe, UK). Nine pasture samples for both periods were all analysed in duplicates, and 135 plasma samples for each period were analysed with 10% of the samples duplicated to check consistency. Enrichment of plasma in $\delta^{15}N$ relative to the diet ($\Delta^{15}N$) was calculated as plasma $\delta^{15}N$ ─ pasture $\delta^{15}N$.

5.2.3 Prediction calculations

Measuring dry matter intake to estimate feed and nitrogen efficiency in field studies is difficult. Long-term experiments involving the measurement of feed intake of cattle are laborious and costly (Archer, 1999). Using back-calculations to calculate dry matter intake can be complex, and several prediction equations have been developed to assist with these estimates (e.g. Vadiveloo & Holmes, 1979; NRC. 1996) however these may not always account for between-animal variation. Measured DMI and NUE were compared with back-calculated measurements in a study by Wheadon et al. (2012) which showed strong correlations between measured and calculated DMI (r = 0.71; P<0.001) and measured and calculated NUE (r = 0.86; P<0.001), suggesting strong power in the use of these equations in the absence of measured intake.

In this study pasture digestible organic matter in the total DM (DOMD) and ME were estimated using equations from MAFF (1984); DOMD (g/kg DM) = 0.98 × DM digested (DMD; g/kg DM) ─ 4.8 and ME (MJ/ kg DM) = DOMD (g/kg DM) × 0.016.

Dry matter intake (kg/day) was estimated by back-calculations using predicted ME requirements for maintenance and milk production from Nicol and Brookes (2007). $ME_{maintenance}$ (MJ/day) = 0.56 MJ ME/kg metabolic live weight ($LWT^{0.75}$); $ME_{lactation}$ (MJ/day) = 1.1 × milk yield (kg/day) × net milk energy ($NE_{lactation}$) divided by the efficiency with which ME is utilised (termed k_l); $ME_{activity}$ = 0.0037 MJ ME/kg LWT per horizontal km walked. $NE_{lactation}$ was calculated from milk composition analysis = (0.376 × fat %) + (0.209 × protein %) + 0.976 MJ NE/litre and k_l = (feed ME MJ/ kg DM × 0.02) + 0.4)). Dry matter intake (kg/day) was then estimated as total ME requirement (MJ/day) divided by feed ME (MJ/kg DM). Nitrogen-use efficiency (g/g) was calculated as milk N (g/cow/day) divided by feed N (g/cow/day).

5.2.4 Statistics

A paired sample t-test was used to compare production and efficiency changes between periods 1 and 2. Two way ANOVA was used to analyse differences between the 9 treatment groups for production, efficiency and plasma $\delta^{15}N$ and $\Delta^{15}N$ using SR and breed group as treatment factors and also the interaction of SR × breed. Initial results showed significant differences between treatment groups for production, efficiency and N isotopes, therefore general linear regression models were used to analyse the relationship between NUE, plasma $\delta^{15}N$ and $\Delta^{15}N$ using NUE as the response and plasma $\delta^{15}N$ or $\Delta^{15}N$ plus treatment group (1-9) as the fitted model. All statistics were conducted in Genstat (13th Ed; VSN international).

5.3 Results

5.3.1 Production and efficiency

Pasture composition for each of the 9 treatment groups for period 1 and 2 is summarised in Table 5.1. Average pasture CP (g/kg DM) and ME (MJ/kg DM) was 192 (s.d. = 2.24) and 222 (s.d. = 0.83) and 11.9 (s.d. = 0.04) and 12.0 (s.d. = 0.29) for periods 1 and 2 respectively.

Table 5.1 Mean composition of pasture (g/kg DM) for the 9 treatment groups for (a) period 1 and (b) period 2.

(a)

Stocking Rate	Breed	DM	CP	DMD*	WSC	DOMD†	ME, MJ/kg DM
High	HF	170	191	768	125	748	11.96
High	J × HF	170	194	765	127	742	11.88
High	J	166	200	758	104	746	11.93
Medium	HF	162	218	766	108	738	11.82
Medium	J × HF	173	184	763	134	741	11.86
Medium	J	162	216	765	92	745	11.92
Low	HF	174	157	763	105	745	11.92
Low	J × HF	169	211	764	104	744	11.91
Low	J	155	154	761	90	743	11.89

(b)

Stocking Rate	Breed	DM	CP	DMD*	WSC	DOMD†	ME, MJ/kg DM
High	HF	161	218	744	102	724	11.58
High	J × HF	152	217	765	59	780	12.49
High	J	151	226	763	59	756	12.10
Medium	HF	163	215	777	90	742	11.88
Medium	J × HF	153	230	790	114	746	11.93
Medium	J	154	235	746	70	726	11.62
Low	HF	162	219	801	135	745	11.91
Low	J × HF	155	207	784	96	764	12.22
Low	J	159	228	766	83	769	12.31

*Dry matter digestibility
†Digestible organic matter on a DM basis

Milk production and composition for periods 1 and 2 is shown in Table 5.2. NUE (g/g) significantly decreased between period 1 (range 0.18 to 0.34) and period 2 (range 0.15 to 0.25). Live weight (kg) did not change between periods 1 and 2. Dry matter intake (kg/d) significantly decreased, whilst feed N (g/d) increased and BCS decreased. Milk yield (kg/d), milk N (g/d) and milk solids (kg/d) significantly decreased between periods 1 and 2 and protein (%) and fat (%) increased.

Table 5.2 Mean production and composition data and s.d. for periods 1 and 2 and associated paired t-test s.e.m and P-value.

	Period 1	Period 2	s.e.m	*P*-value
LWT, kg	479	478	1.83	n.s
DMI, kg/d	13.8	12.8	0.09	<0.001
Feed N, g/d	422	453	5.85	<0.001
Condition score	2.84	2.78	0.02	<0.001
Milk yield, kg/d	17.2	14.7	0.16	<0.001
Protein, %	3.77	3.99	0.15	<0.001
Fat, %	4.54	4.89	0.05	<0.001
Milk solids, kg	1.40	1.28	0.01	<0.001
Milk N, g/kg	100	91	0.97	<0.001
NUE, g/g	0.24	0.20	0.03	<0.001

5.3.2 Isotope measurements

Average plasma $\delta^{15}N$ for periods 1 and 2 was 7.55 ‰ (s.d. = 0.30) and 7.55 ‰ (s.d. = 0.30) respectively. There was a significant correlation between plasma $\delta^{15}N$ for individual cows in periods 1 and 2 (Figure 5.1).

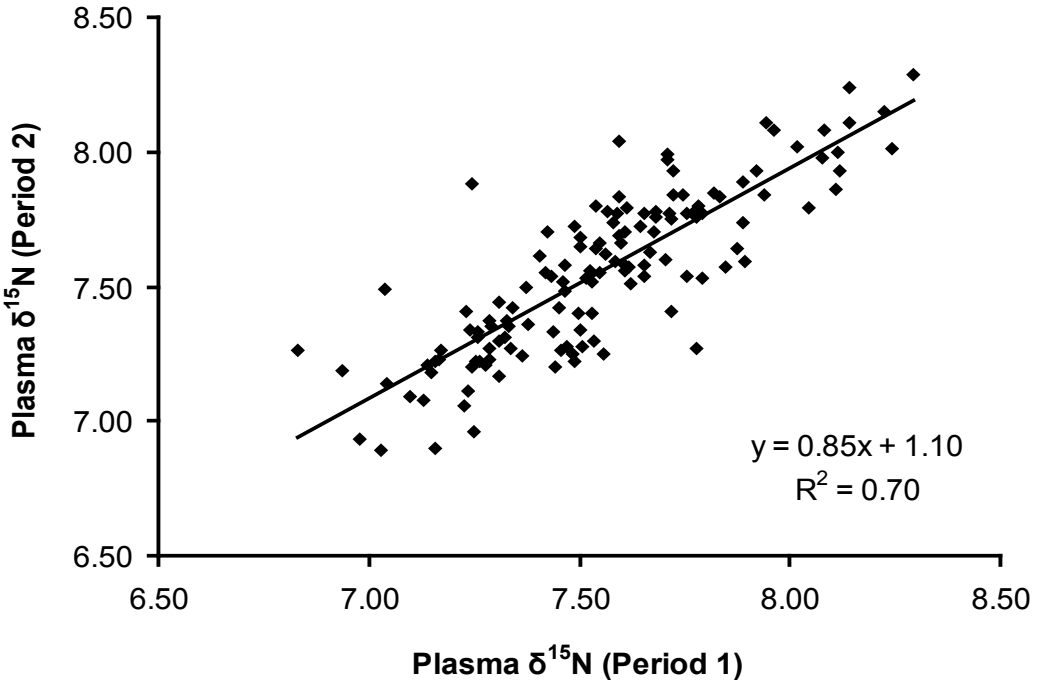


Figure 5.1 Relationship between plasma $\delta^{15}N$ (‰) in periods 1 and 2 for individual cows.

Mean pasture $\delta^{15}N$ was 3.62 ‰ (s.d. = 0.50) and 4.84 ‰ (s.d. = 0.48) in periods 1 and 2 respectively, resulting in higher average enrichment ($\Delta^{15}N$; plasma $\delta^{15}N$ – pasture $\delta^{15}N$) in period 1 (3.92 units; s.d. = 0.50) than period 2 (2.71 units; s.d. = 0.49) (Figure 5.2).

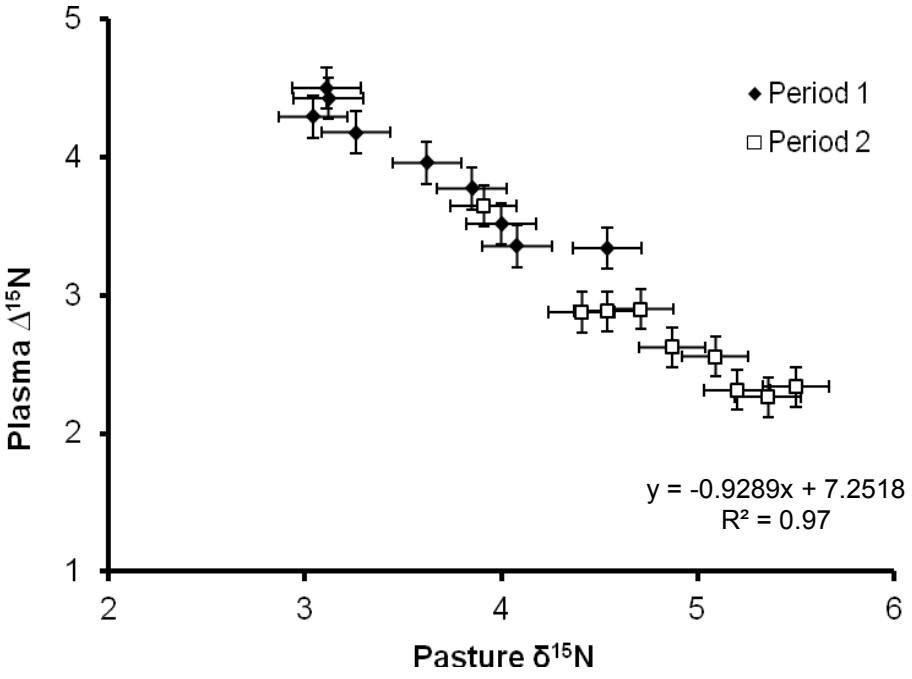


Figure 5.2 Relationship between pasture $\delta^{15}N$ (‰) and plasma $\Delta^{15}N$ for periods 1 and 2 for treatment group means.

There was a positive correlation between pasture CP (g/kg DM) and pasture $\delta^{15}N$ for both periods ($P<0.001$; $r^2 = 0.55$) (Figure 5.3). There was higher variation in CP in period 1 compared to period 2. In period 1, CP ranged from 15.1 to 21.8% and in period 2 it ranged from 20.7 to 23.5%. There were also positive correlations between plasma $\delta^{15}N$ and pasture $\delta^{15}N$ ($r = 0.30$; $P<0.001$) and ($r = 0.29$; $P<0.001$) in periods 1 and 2 respectively.

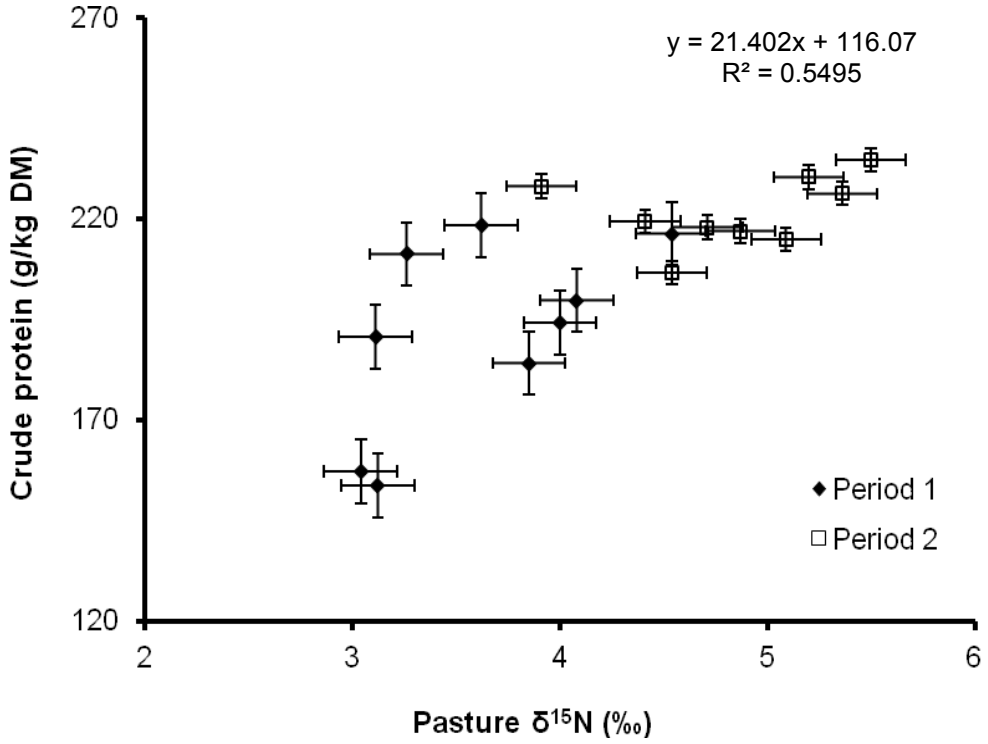


Figure 5.3 Relationship between pasture crude protein (g/kg DM) and pasture $\delta^{15}N$ (‰) for treatment means for periods 1 and 2 for treatment group means

5.3.3 Treatment effects

Milk production and feed efficiency estimates for the 9 treatment groups for periods 1 and 2 are summarised in Table 5.3. In period 1, animals in low SR groups had the highest DMI (kg/d), milk solids (kg/d), milk N (g/d) and NUE (g/g). Holstein-Friesian cows had the highest LWT (kg), DMI (kg/d), milk yield (kg/d) and milk N (g/d) and Jersey cows had the lowest.

In period 2, animals in low SR groups had the highest DMI (kg/d), milk yield (kg/d), milk solids (kg/d), milk N (g/d) and NUE (g/g). Holstein-Friesian cows had the highest LWT (kg), DMI (kg/d) and milk yield (kg/d) and Jersey cows had the lowest. The interaction of SR and breed also had a significant effect on NUE (g/g) in both periods 1 and 2.

Table 5.3 Mean production and efficiency for the 9 treatment groups and ANOVA significance for (a) period 1 and (b) period 2

(a)

Stocking Rate	Breed	LWT kg	DMI kg/d	Milk yield kg/d	Milk solids kg/d	Milk N g/kg	NUE g/g
High	HF	523	13.8	18.1	1.32	97	0.23
High	J × HF	476	13.2	16.5	1.32	95	0.23
High	J	392	11.6	13.3	1.20	84	0.23
Medium	HF	561	14.8	19.4	1.39	107	0.21
Medium	J × HF	499	14.7	18.4	1.51	109	0.25
Medium	J	397	12.8	14.3	1.42	94	0.21
Low	HF	573	15.1	20.1	1.45	109	0.29
Low	J × HF	479	14.8	19.1	1.57	114	0.23
Low	J	414	13.4	15.5	1.46	97	0.29
s.e.d							
SR		11.1	0.37	0.63	1.39	3.42	0.004
Breed		11.1	0.37	0.63	1.36	3.42	0.004
SR × Breed		19.3	0.64	1.10	1.44	5.92	0.006
P- value							
SR		<0.1	<0.001	<0.01	<0.001	<0.001	<0.001
Breed		<0.001	<0.001	<0.001	<0.1	<0.001	n.s
SR × Breed		n.s	n.s	n.s	n.s	n.s	<0.001

(b)

Stocking Rate	Breed	LWT kg	DMI kg/d	Milk yield kg/d	Milk solids kg/d	Milk N g/kg	NUE g/g
High	HF	549	13.1	15.0	1.10	82	0.18
High	J × HF	456	11.2	13.1	1.14	82	0.21
High	J	382	10.7	11.5	1.11	77	0.20
Medium	HF	540	13.7	16.2	1.29	94	0.20
Medium	J × HF	477	13.5	16.4	1.38	104	0.21
Medium	J	414	12.4	12.4	1.26	85	0.18
Low	HF	579	14.1	17.3	1.30	98	0.20
Low	J × HF	487	13.9	16.7	1.53	108	0.23
Low	J	421	12.5	13.3	1.42	90	0.20
s.e.d							
SR		10.4	0.31	0.50	0.04	3.01	0.003
Breed		10.4	0.31	0.50	0.04	3.01	0.003
SR × Breed		18.0	0.54	0.86	0.08	5.21	0.006
P- value							
SR		<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
Breed		<0.001	<0.001	<0.001	<0.05	<0.001	<0.001
SR × Breed		n.s	n.s	n.s	n.s	n.s	<0.001

There were also significant differences between plasma $\delta^{15}N$ and $\Delta^{15}N$ for the 9 treatment groups (Table 5.4); however there was a small range in plasma $\delta^{15}N$ between groups which was higher for period 2 (1.59 ‰) than period 1 (0.97 ‰). In both periods 1 and 2, $\Delta^{15}N$ was highest for animals in the high SR groups and in Jersey cows.

Table 5.4 Mean pasture and plasma $\delta^{15}N$ (‰) and $\Delta^{15}N$ for the 9 treatment groups and ANOVA significance for periods 1 and 2

Period		1			2		
Stocking Rate	Breed	Pasture $\delta^{15}N$	Plasma $\delta^{15}N$	$\Delta^{15}N$	Pasture $\delta^{15}N$	Plasma $\delta^{15}N$	$\Delta^{15}N$
High	HF	3.11	7.61	4.50	4.71	7.61	2.90
High	J	3.62	7.58	3.96	5.09	7.65	2.56
High	J × HF	3.04	7.33	4.29	4.41	7.23	2.88
Medium	HF	4.08	7.44	3.36	5.36	7.62	2.26
Medium	J	4.54	7.88	3.34	5.50	7.81	2.34
Medium	J × HF	3.12	7.55	4.43	3.91	7.56	3.65
Low	HF	4.00	7.52	3.52	4.87	7.49	2.62
Low	J	3.85	7.62	3.77	5.20	7.51	2.31
Low	J × HF	3.26	7.44	4.18	4.54	7.43	2.89
s.e.d							
SR			0.06	0.06		0.06	0.06
Breed			0.06	0.06		0.06	0.06
SR × Breed			0.10	0.10		0.10	0.10
P- value							
SR			<0.1	<0.001		<0.01	<0.01
Breed			<0.001	<0.001		<0.001	<0.001
SR × Breed			<0.01	<0.001		n.s	<0.001

5.3.4 Relationship between N isotopic fractionation and NUE

There was no significant relationship between NUE and plasma $\delta^{15}N$ or $\Delta^{15}N$ after taking account of the effect of treatment group in the regression analyses. For period 1, the mean slope of the regression equation was -2.16 (s.e. = 1.41) and the intercept was 8.11 (s.e. = 0.33) for plasma $\delta^{15}N$ and 5.00 (s.e. = 0.33) for $\Delta^{15}N$. For period 2, the mean slope of the regression equation was -1.95 (s.e. = 1.57) and the intercept was 7.96 (s.e. = 0.29) for plasma $\delta^{15}N$ and 3.25 (s.e. = 0.29) for $\Delta^{15}N$.

5.4 Discussion

5.4.1 Pasture composition and $\delta^{15}N$

In this study, the higher pasture CP in period 2 (mean 22.2 %) than in period 1 (mean 19.2%) resulted in an increase in the excess of N not required by the animal (recommended requirements = 16% CP (NRC, 2001). As pasture $\delta^{15}N$ was positively correlated to CP, it was also was higher in period 2. They were likely correlated because fertiliser application and sward grazing height may have resulted in paddocks being fertilised at different times, which created differences in the pool of N in the soil when samples were taken. This affects the sources of N for pasture growth, which depends on fixation of atmospheric N_2 by bacteria, oxidation of ammonia to nitrate (Delwiche and Steyn, 1970) and denitrification (Wellman et al. 1968) all of which have different isotopic fractionation effects (Rennie et al. 1976).

5.4.2 Production and efficiency

In this study, NUE decreased by an average 4% in period 2 because there was higher CP in pasture in period 2 (section 5.4.1), and because there may have been a change in the animals' physiological state. Cows in period 1 also had significantly higher milk yield and intake compared to period 2, so there would have been higher dilution of maintenance costs for increased milk output in period 1. There was no change in LWT between periods because samples were collected during mid-lactation when

there are low fluctuations in body weight from the mobilisation of body reserves compared to early lactation (Friggens et al., 2004; Khan et al., 2013).

5.4.3 Breed and stocking rate effects

There were significant differences in production and efficiency between the treatment groups, partly caused by differences in diet composition, but also because of breed and SR differences. Dairy farmers in Ireland aim to maximise milk solids per hectare because high SR may maximise profitability despite not maximising individual animal performance. Despite this, results in this study suggest that high SR groups were not the most N efficient. However, there was a small range in NUE across treatment groups (8% in period 1 and 5% in period 2) so higher observed efficiency for the low SR groups was likely because of lower CP in the pasture. Groups of cows with CP levels closer to their requirements (16 %; NRC, 2001) had higher NUE because they wasted less to urine. Therefore, high SR groups would have been more efficient if pasture contained less CP.

Jersey cows had lower LWT, intake and milk production than Holstein-Friesian cows and cross breeds across all SR groups. In comparison, Holstein-Friesian cows had the highest milk production and intake. Holstein-Friesian × Jersey cows were the most N efficient in this study, followed by Jersey pure breeds and pure Holstein-Friesians had the lowest NUE. Previous research has suggested that Jersey cows are the most feed efficient breed, and are consistently highest over the lactation cycle (i.e. show less change in body condition score) (Prendiville et al., 2009, 2011). There has been greater profit in Jersey cross-breeding because of their easier management (Lopez-Villalobos et al., 2000; Grainger and Goddard, 2007) and the increased performance of cross breeds above the mid-parent mean from hybrid vigour (Begley et al., 2009).

5.4.4 Nitrogen isotopic fractionation and NUE

Enrichment of plasma ($\Delta^{15}N$) averaged 3.92 ‰ (s.d. = 0.50) and 2.71 (s.d. = 0.49) in periods 1 and 2 in this study. Values for period 2 were similar to results in previous studies with lactating animals e.g. $\Delta^{15}N$=2.37 (Sutoh et al., 1987) $\Delta^{15}N$=2.68 (Cheng et al., 2011), however for period 1, $\Delta^{15}N$ was higher and more similar to studies involving growing beef cattle e.g. $\Delta^{15}N$=4.2 (Steele and Daniel, 1978) $\Delta^{15}N$= 4.0 (Sponheimer et al., 2003). The difference in enrichment of plasma was a result of higher pasture $\delta^{15}N$ in period 2. However, we do not fully understand the reason for this difference but suspect it may be a result of the increase in pasture CP in period 2. This would suggest that pasture $\delta^{15}N$ fluctuates more rapidly over time compared to plasma $\delta^{15}N$. Plasma $\delta^{15}N$ would be more representative of pasture consumed over a longer period of time than the ^{15}N enrichment of a single forage sample collected on the same day as plasma.

Plasma $\delta^{15}N$ was similar between periods and there was a small range between the treatment groups, which was greater for period 2 (1.59 ‰) than period 1 (0.97 ‰). As a result, $\Delta^{15}N$ was greater in period 2 but differences in groups were mainly the result of changes in pasture $\delta^{15}N$, which was correlated with pasture CP, and not plasma $\delta^{15}N$. Plasma $\delta^{15}N$ within individuals was highly correlated between period 1 and 2 (Figure 5.1) because there were very small changes in efficiency within groups over time. These findings are consistent with highly correlated plasma $\delta^{15}N$ measurements over 4 time points in growing beef heifers on the same diet in Wheadon et al. (2014). Plasma also has a slower incorporation of protein than high turnover tissues such as milk (Waterlow, 1984; Boldt, 2010) so isotope signatures do not change as rapidly.

5.4.4.1 Excess dietary nitrogen and nitrogen isotopic fractionation

The dominant factor determining NUE is N intake, which is likely to be highly related to excess RDP. An industry wide problem is that good management of pasture yield often leads to high herbage N content, which leads to a higher intake of CP and RDP and N in either form in excess of animal requirements decreases NUE and increases the fractionation of N isotopes due to the increased excretion of urea in urine. Therefore, it is difficult to say to what extent the differences in NUE were attributable to differences in RDP intake versus N intake. MP is used efficiently provided that it is close to the requirement of the animal and that there is enough energy in the diet. An excess of MP relative to energy is as detrimental as an excess of RDP in the rumen.

In order to provide evidence of the effects of excess RDP to N inefficiency, estimates of RDP and microbial protein yield were made using equations and values from AFRC (1992) based on the assumptions that in this case all RDP was excreted as urea and not recycled. Rumen degradable protein exceeded requirements by 19% (s.d. = 0.08) and 29% (s.d. = 0.03) in periods 1 and 2 respectively. This would account for 25% (s.d. = 0.10) and 36% (s.d. = 0.03) of N not incorporated into milk N in periods 1 and 2 respectively. Lower levels of excess RDP were associated with more N efficient animals in both periods (Figure 5.4).

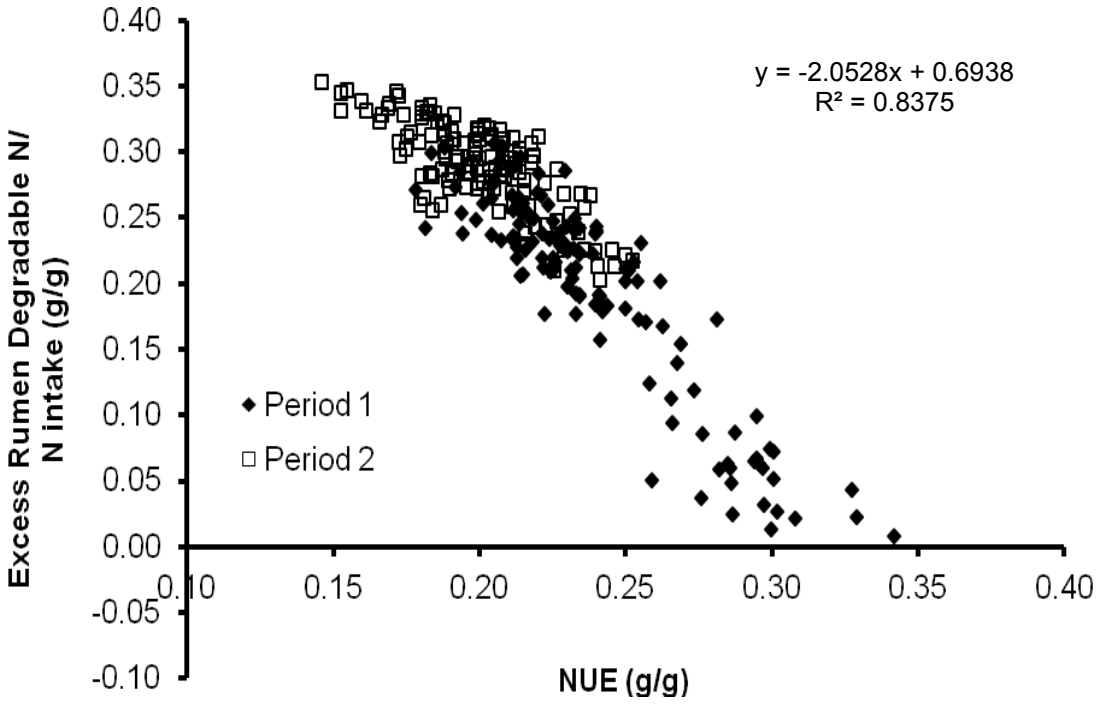


Figure 5.4 Relationship between NUE (g/g) and excess rumen degradable N/N intake (g/g) for period 1 and period 2 for individual cows.

There was a large range in the excess RDP between groups in period 1 (1 to 31%), which relates to the greater variation in herbage N content and explains the higher NUE (g/g) in low SR groups as they had lower CP (mean 174 g/kg DM) than medium SR (mean 206 g/kg DM) and high SR (mean 195 g/kg DM) groups. In contrast, CP (and excess RDP) was higher and more similar over SR groups (range 20 to 35 %) in period 2, so the range in NUE was smaller. In the case of increasing dietary N, an increasing proportion of N was directed to urine so fractionation rate increased, causing a higher isotopic signature for the groups with higher CP. There is increased urea synthesis either from catabolism of amino acids absorbed in excess of requirement, or excess ammonia absorption (both due to higher N intakes) which causes higher tissue enrichment of $\delta^{15}N$ relative to the diet.

There were weak relationships between NUE and N isotope fractionation in this study, which may be explained by 3 main factors, (i) the inclusion of 9 treatment groups which caused variation in relationships, (ii) a narrow range in NUE and plasma $\delta^{15}N$ which made it difficult to detect between-animal variation and (iii), the large influence of excess N above animal requirements caused an increase in the excretion of urea and subsequently N fractionation rate (particularly in period 2). This suggests that $\Delta^{15}N$ is driven by N metabolism in animal tissues and NUE is driven by rumen efficiency. The excess RDP was not incorporated into microbial protein, and so was not associated with isotopic fractionation in this study.

5.5 Conclusion

In this study there were significant differences in production and N efficiency for cows grazed on different SR and differing in breed. There were weak relationships between NUE and N isotopic fractionation likely because of the effects of the 9 treatment groups and because there was a narrow range in NUE and plasma $\delta^{15}N$. In addition there was an excess of N in the pasture leading to an excess of RDP that would not be associated with isotopic fractionation. The excess of RDP in the diet may be a limitation in the use of isotope fractionation as a marker for NUE with diets high in N, which are often the case in New Zealand. There was unexpected variation in protein in pasture in this study; however there would still be value in measuring isotopic fractionation to assess NUE when animals are fed contrasting diets, if it can be used as a tool to distinguish which diet leads to a better NUE. Initially, measuring between-animal variation driven by genetics in N partitioning and N isotopic fractionation would be more suitable using animals on the same management and diet to eliminate group effects. Diets high in N may compromise the use of the N fractionation approach to predict NUE and requires further investigation.

CHAPTER 6

The effects of breeding worth and sire on nitrogen-use efficiency and nitrogen isotope fractionation in dairy cows on pasture

6.1 Introduction

Metabolisable protein consists of amino acids absorbed from the small intestine, following post-ruminal digestion of microbial protein, and UDP. Absorbed amino acids are then available for maintenance, lactation and growth (Brookes and Nicol, 2007). There are three main components of protein metabolism in ruminants: (i) microbial degradation and synthesis in the rumen, (ii) absorption in the small intestine and (iii) catabolism and excretion of excess digestible protein into faeces and urine N (ARC, 1980). Inefficiency in the use of dietary protein results both from inefficient conversion of RDP into microbial protein and inefficient use of absorbed amino acids for milk protein synthesis (Lapierre et al., 2005).

There has been little selection for feed efficiency traits (Ngwerume and Mao, 1992) because it requires the development of robust phenotype markers to be useful for large numbers of animals (Zamani et al., 2011). In this study, we considered N isotope fractionation as an indicator of NUE. Isotope fractionation has been used as a tool to understand N partitioning in archaeological and ecological research (Weast, 1983; Sponheimer et al., 2003; Nardoto et al., 2006). Kinetic isotope fractionation occurs in incomplete or branching reactions due to differences in rates of reactions of compounds containing heavier or lighter isotopes (Macko et al., 1986, 1987; Kendall and Caldwell, 1998). Animals are more enriched in $\delta^{15}N$ than their diet (termed $\Delta^{15}N$; animal tissue $\delta^{15}N$ – diet $\delta^{15}N$) (Ambrose and De Niro, 1986). This approach has recently been investigated in dairy cattle (Cheng and Dewhurst, 2009; Cheng et al., 2011) which has shown consistency in the enrichment of $\delta^{15}N$ in milk protein, and a negative relationship between $\Delta^{15}N$ (milk) and NUE in Holstein-Friesian cows. In ruminants, most fractionation is likely to occur in liver tissues. Transamination and deamination during urea synthesis causes ^{14}N in feed to be preferentially utilised,

resulting in a depletion of ^{15}N in urea and ammonia formed in the liver, and enrichment of proteins compared to the amino acids of the free metabolic pool (Sick et al., 1997).

Breeding Worth (BW; New Zealand Animal Evaluation Limited, New Zealand) is used to rank animals that are most efficient at creating profit and high value replacements and is calculated by combination of the economic worth of important traits (milk fat, protein, milk volume, LWT, fertility, somatic cells and residual survival). Production worth (PW) and lactation worth (LW) are related indices that rank females for their lifetime production ability and their current season production ability.

It has been reported that there are genetic differences between animals with different BW in respect to energy metabolism and production (Woodward et al. 2011). High BW cows have higher feed efficiency (Davey et al., 1983) therefore we would predict differences in N partitioning, which is consistent with findings from McPherron and Lee (1997) and preliminary findings of differences in NUE between high and low BW cows (Wheadon et al., 2013). Identifying between-animal variation is an important aspect to the genetic selection of animals that are divergent for N utilisation. The Breeding Worth indexes (New Zealand Animal Evaluation Limited, New Zealand) do not directly select for feed efficiency components or traits, however they do select for aspects such as increased milk protein and milk yield, which may help to identify variation in efficiency in groups of animals.

In the previous chapter, there were weak relationships between NUE and N isotopic fractionation which was partially due to the inclusion of 9 treatment groups and also due to the excess of RDP that was measured in the pasture; the excess of N in the diet may be a limitation of the use of isotope fractionation as a marker of NUE with diets with high N. In this study the first objective was to investigate the relationship between N isotope fractionation ($\delta^{15}N$) and NUE in dairy cows on pasture that were not in treatment groups. The second objective was to investigate differences in

production, efficiency and N isotope fractionation in groups of daughters with different sires and animals differing in BW.

6.2 Materials and methods

6.2.1. Preparation methods

Sampling is important to consider when measuring N isotope fractionation because $\delta^{15}N$ is a sensitive technique. Using dried, ground samples of milk has been commonly used for preparation, however this is more time consuming than using liquid samples. Therefore a preliminary study was conducted prior to sample collection and analysis in this study to test two methods of preparation of milk for Isotope Ratio Mass Spectrometry (IRMS). In addition, the relationship between $\delta^{15}N$ for samples collected at am and pm milking was investigated. Sub samples for the comparison of $\delta^{15}N$ for am (n=20) and pm (n=20) milk were taken directly from chilled milk samples (20µl). This method was used to further sub sample pm milk (n=20) and a repeated set that were heated in a water bath to 20^{o}C before mixing and sub sampling (n=20). All samples were then oven dried (24 hours at 40^{o}C) in foil capsules (8mm × 5mm) and sent for analysis for the ratio of ^{14}N to ^{15}N (IsoAnalytical, Crewe, U.K), reported in delta units ($\delta^{15}N$).

6.2.2 Sampling

Experiments were conducted at the Lincoln University Dairy Farm (Lincoln, Canterbury, New Zealand); a 186 hectare irrigated 620 cow seasonal supply commercial farm with an emphasis on sustainable farming with high quality pasture and high productivity. Sire breeds were pure bred Jersey or Jersey × Holstein-Friesian and thirty-seven sires accounted for 80% of daughters in the whole herd. Parity ranged from 1 to 8 and BW (NZ$) ranged from -24 to 226.

Cows were milked twice daily; duplicate milk samples (2 samples at the pm milking time) were collected from a subset of cows at 2 time points in mid-lactation. Period 1

samples were collected on December 12th 2011 from 200 cows; mean calving date was 30th June 2011 and days in milk was 165 (s.d. = 20). Period 2 samples were collected on February 15th 2012 from 550 cows; mean calving date was 17th August 2011 and mean days in milk was 181 (s.d. = 20).

Cows were rotationally grazed on perennial ryegrass/white clover paddocks. Duplicate pasture samples were collected by hand in a random sampling technique in the paddock, consisting of 10 sub-samples collected in different areas and combined to create the overall sample for the paddock. These were collected on the day prior to milk sampling from the paddock cows were due to move to the same day, and fresh samples were sent for composition analysis (Analytical Research Laboratories Ltd; Napier, New Zealand). Pasture was analysed for dry matter (%) by drying at 105ºC for 24 hours. Pasture was analysed by NIRS for crude protein (% DM) calcuated from total nitrogen, lipid (% DM), ash (% DM), ADF (% DM), NDF (% DM), OMD (in vivo, % DM) and ME (estimated) MJ/kg DM. NIRS calibration was maintained by reference to wet chemistry methods.

Milk was analysed by NIRS (NIRS; Livestock Improvement Corporation, Hornby, New Zealand) for composition of protein, fat, lactose and somatic cell count. Sub-samples of milk and pasture were also analysed by Isotope Ratio Mass Spectrometry by measuring 20mg of freeze-dried milk for each cow into individual tin capsules (PDZ Europa 20-20, Lincoln Analytical services, Lincoln University), for the ratio of ^{14}N to ^{15}N, reported in delta units ($\delta^{15}N$; ‰) and enrichment of milk ^{15}N termed $\Delta^{15}N$ (milk $\delta^{15}N$ ─ pasture $\delta^{15}N$). Animal LWT and milk yield was recorded at the milk shed by using automatic recording systems.

6.2.3 Prediction calculations

Dry matter intake (kg/day) was estimated by back-calculation using equations from Nicol and Brookes (2007). Predictions were made by calculating ME requirements for maintenance: $ME_{maintenance}$ (MJ/day) = 0.56 MJ ME/kg metabolic LWT ($LWT^{0.75}$), plus ME requirements for lactation: $ME_{lactation}$ (MJ/day) = 1.1 × milk yield (kg/day) × net milk energy ($NE_{lactation}$) divided by the efficiency with which ME is utilised (termed k). $NE_{lactation}$ = (0.376 × fat %) + (0.209 × protein %) + 0.976 MJ NE/litre and k = (feed ME MJ/ kg DM × 0.02) + 0.4), plus activity requirements (walking): $ME_{activity}$ = 0.0037 MJ ME/kg LWT per horizontal km walked. Dry matter intake (kg/day) was then estimated as total ME requirement (MJ/day) divided by feed ME (MJ/kg DM). Predictions of NUE (g/g) were made by dividing feed N (g/cow/day) by milk N (g/cow/day).

Metabolisable protein supply was estimated using back calculations from Brookes and Nicol (2007): Metabolisable Protein supply (g/day) = (0.64 × microbial protein) + (0.9 × (undegradable dietary protein (UDP) – (Acid detergent insoluble N fraction (ADIN); g/kg DM) × 6.25))) × DMI (kg/day). Microbial protein was estimated by; microbial protein = (7 + (6 × (1-EXP (-0.35 × level of feeding)))) × fermentable ME (MJ/kg DM). Level of feeding = Multiple of maintenance (Total ME/ME maintenance). Undegraded dietary protein was estimated as: CP % × (1- quickly degraded protein (QDP) + slowly degraded protein (SDP)) × 10. Protein degradation values for pasture (QDP, SDP and ADIN) were based on data sourced from AFRC (1993), Burke et al. (2000), Chaves et al. (2001) and Chaves et al. (2002); the value used for QDP (soluble protein fraction) was 0.53 g/g CP, and ADIN was 0.30 g/kg DM (taken from Brookes and Nicol, 2007). Slowly degraded protein was estimated using the insoluble degradable fraction (0.42 g/g CP), fractional protein disappearance rate (14%/hour) and calculating rumen retention time. For additional calculation details refer to Brookes and Nicol. (2007). Metabolisable Protein efficiency (MPE) (g/g) was estimated as milk protein (g/d) divided by MP supply (g/d).

6.2.4 Statistics

Sire groups were formed by using confirmed parentage from DNA testing (GeneMark DNA profile, Livestock Improvement Corperation), consisting of 10 groups which ranged from 6-12 daughters in each group with the same sire (total n=86). Sire groups used were the same for period 1 and 2. BW groups were formed by ranking animals in period 2 into quintiles (n=100 per group); low (BW range -24 to 75), medium-low (BW range 75 to 100), medium (BW range 100-120), medium-high (BW range 120 to 142) and high (BW range 142 to 226).

All statistics were conducted in Genstat (13th Ed; VSN International). Pearson correlations were used to compare the relationship between milk $\delta^{15}N$ collected at am and pm milking and prepared by chilled or heated methods. Linear regression was used to analyze the relationship between NUE and $\delta^{15}N$ and $\Delta^{15}N$ for all animals in periods 1 and 2. REML mixed models were used to analyze differences between sire groups, using sire group as the fixed model and parity plus percentage Jersey of the dam as the random model. ANOVA was used to compare BW groups and describe the polynomial regression model to show the level of curvilinearity (described as linear or quadratic) for the relationship between the BW groups and variables. Pearson correlations were also used to analyze the relationship between NUE and BW, PW and LW.

6.3 Results

6.3.1 Preparation methods

There was a highly significant relationship between milk $\delta^{15}N$ collected at am and pm milking (r = 0.84; P<0.001), and milk prepared by chilled or heated methods for IRMS (r = 0.97) (Figure 6.1). IRMS was not affected by heating the milk which allows the milk fat (that separates when standing) to mix with the rest of the sample. Therefore, the chilled preparation technique was used for subsequent analysis in this study as it is less time consuming, especially with a large number of samples as chilled milk can be directly pipetted into foil capsules.

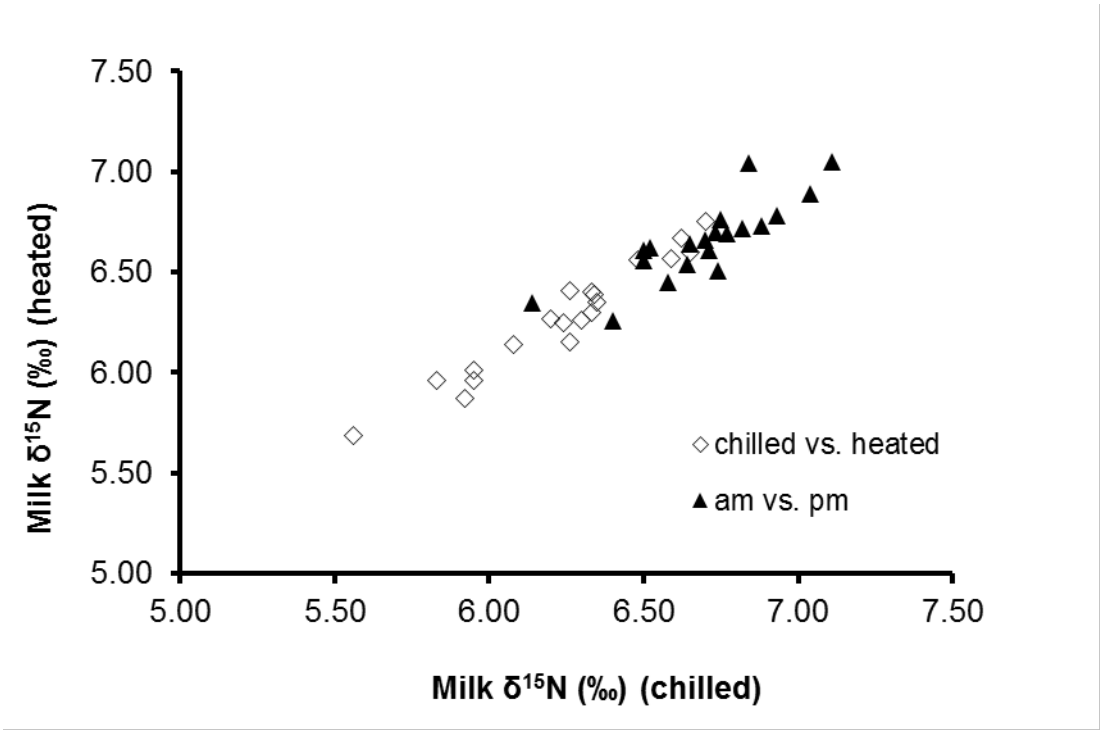


Figure 6.1 Relationship between milk $\delta^{15}N$ prepared for IRMS using chilled or heated methods and samples taken at am and pm milking

6.3.2 Pasture, production and efficiency

Pasture composition for periods 1 and 2 is summarised in Table 6.1; in both cases, CP was very high. Pasture sub-samples from period 1 were lost during preparation therefore $\delta^{15}N$ was measured in period 2 only. Pasture $\delta^{15}N$ was 6.42‰ so average enrichment ($\Delta^{15}N$; milk $\delta^{15}N$ ─ pasture $\delta^{15}N$) was 0.64 (s.d. = 0.44) and ranged from -0.75 to 2.14 units.

Table 6.1 Chemical composition (% of DM, unless otherwise stated) and pasture $\delta^{15}N$ for periods 1 and 2.

	Period 1	Period 2
DM, %	19.4	12.9
CP	25.6	29.8
Lipid	4.20	4.50
Ash	12.1	11.7
ADF	24.5	24.4
NDF	45.0	48.8
OMD (in vivo)[1]	84.3	84.4
ME, MJ/kg DM	12.4	12.1
Pasture $\delta^{15}N$, ‰	ND*	6.42

*Pasture was not analysed in period 1 due to lost samples
[1]OMD (in vivo) % w/w DM was predicted by NIRS.

Production, composition and isotope data for periods 1 and 2 is summarised in Table 6.2. There was a decrease in milk yield (kg/d), increase in LWT (kg) and no change in DMI (kg/d) from periods 1 to 2. There was an increase in feed N (g/d) in period 2 because pasture CP was 4.2% units higher than in period 1. NUE (g/g) and MPE (g/g) both decreased on average by 4% and milk $\delta^{15}N$ also decreased.

Table 6.2 Average production and composition data and associated s.d. for all animals in periods 1 (n=200) and 2 (n=550)

	Period 1	s.d.	Period 2	s.d.
BW, $	106	34	108	38
PW, $	134	73	140	84
LW, $	158	123	161	147
LWT, kg	466	51	484	53
DMI, kg/d	14.9	2.1	14.9	2.8
Milk yield, kg/d	19.7	4.3	15.8	4.0
Fat, %	5.10	0.69	6.49	1.21
Protein, %	3.83	0.31	4.37	0.61
Lactose, %	5.11	0.14	5.11	0.59
Milk solids, kg/d	1.73	0.30	1.72	0.43
Milk N, g/d	116	20	109	25
Feed N, g/d	607	84	707	133
FCE, g/g	0.12	0.01	0.11	0.01
NUE, g/g	0.19	0.01	0.15	0.02
MPE, g/g	0.40	0.02	0.36	0.03
Milk $\delta^{15}N$, ‰	7.28	0.50	7.06	0.44
$\Delta^{15}N$	ND		0.64	0.44

6.3.3 Relationship between NUE and N isotopic fractionation

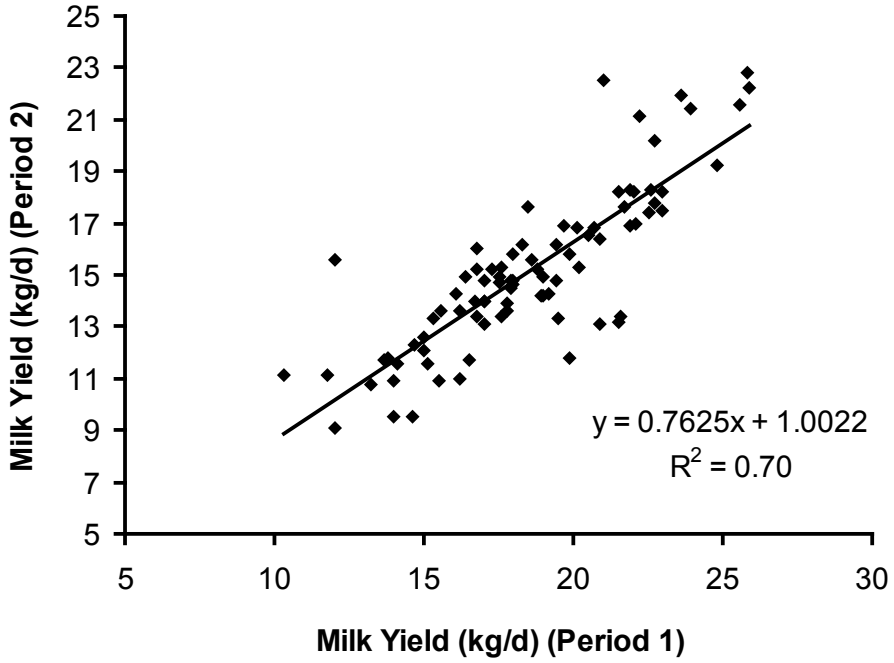
There was no significant relationship between NUE and milk $\delta^{15}N$ in period 1 and a weak negative relationship between NUE and both milk $\delta^{15}N$ ($P<0.1$) and $\Delta^{15}N$ ($P<0.1$) in period 2. Adding the sire, sire breed or parity (1-8) as factors in the regression model explained additional significant parts of the variation in milk $\delta^{15}N$, however there was still high variation (mean $r^2= 0.10$).

6.3.4 Sire groups

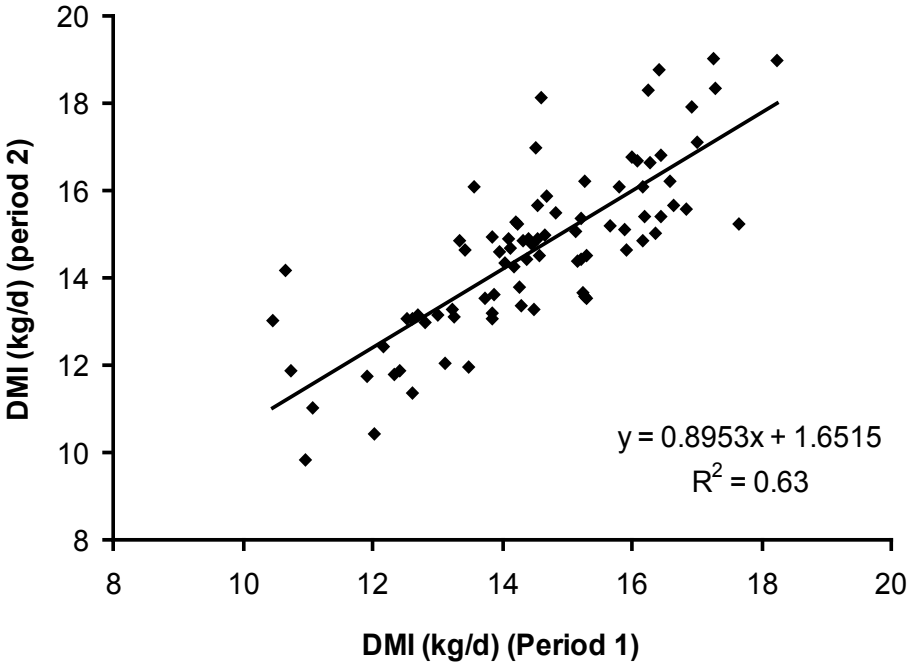
6.3.4.1 Relationship between daughters in periods 1 and 2

There were significant correlations between milk yield (kg/d), DMI (kg/d), milk protein (g/d) and NUE (g/g) for individual daughters in the sire groups measured in periods 1 and 2 (Figure 6.2 (a)-(d)). However, there was no correlation between values for milk $\delta^{15}N$ (Figure 6.2 (e)).

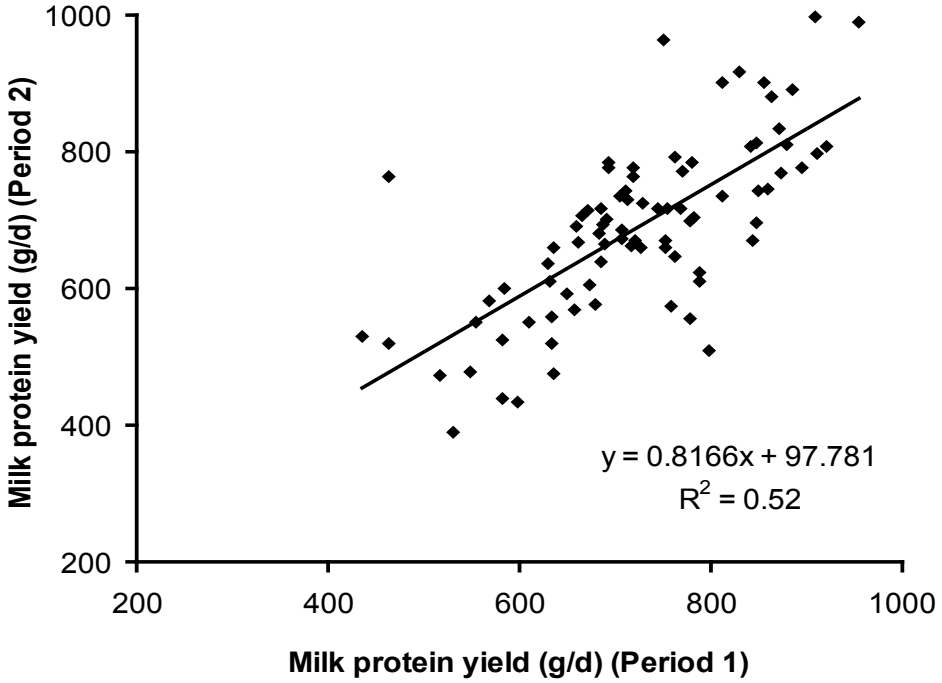
(a)


Milk Yield (kg/d) (Period 2)
Milk Yield (kg/d) (Period 1)
y = 0.7625x + 1.0022
R2 = 0.70
25
23
21
19
17
15
13
11
9
7
5
5
10
15
20
25
30

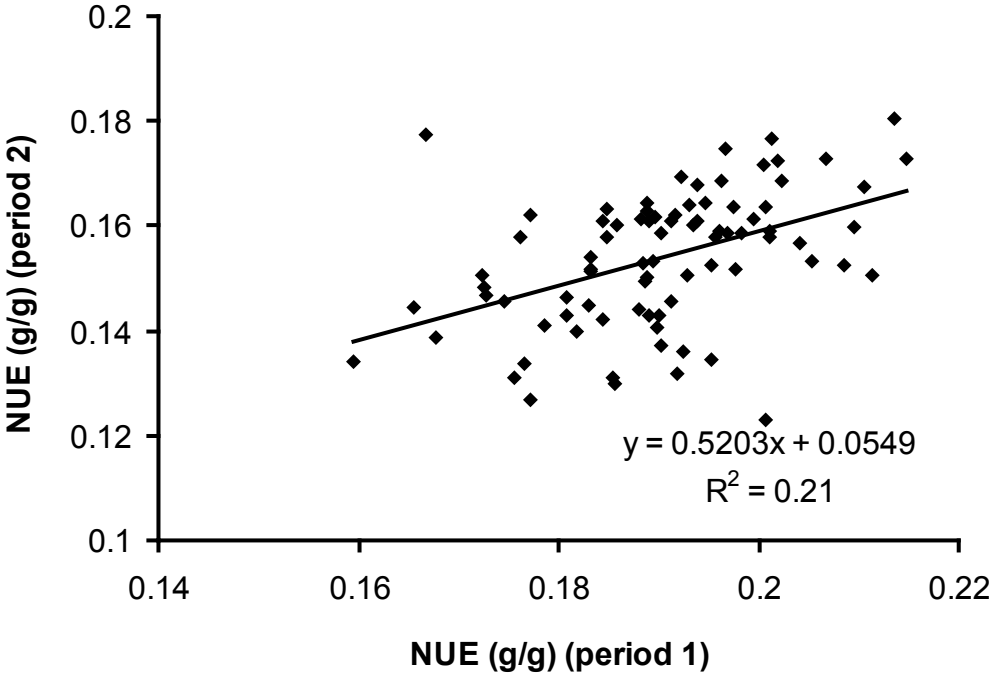
(b)


DMI (kg/d) (period 2)
DMI (kg/d) (Period 1)
y = 0.8953x + 1.6515
R2 = 0.63
20
18
16
14
12
10
8
8
10
12
14
16
18
20

(c)


Milk protein yield (g/d) (Period 2)
1000
800
600
400
200
200
400
600
800
1000
y = 0.8166x + 97.781
R2 = 0.52
Milk protein yield (g/d) (Period 1)

(d)


NUE (g/g) (period 2)
0.2
0.18
0.16
0.14
0.12
0.1
0.14
0.16
0.18
0.2
0.22
y = 0.5203x + 0.0549
R2 = 0.21
NUE (g/g) (period 1)

(e)

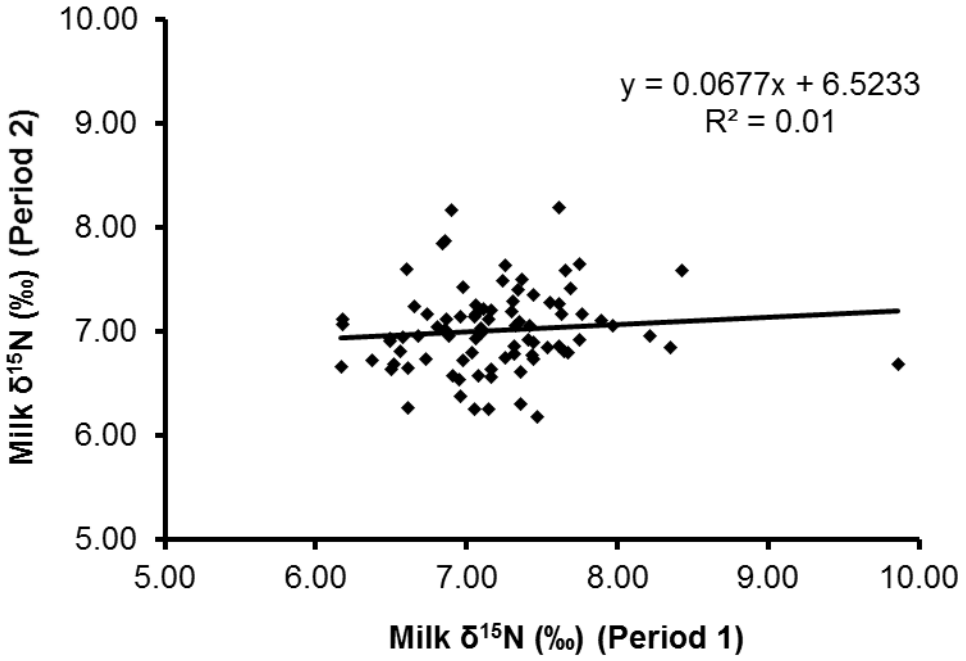


Figure 6.2 The relationship between individual measurements of (a) milk yield (kg/d), (b) DMI (kg/d), (c) milk protein yield (g/d), (d) NUE (g/g) and (e) milk $\delta^{15}N$ in periods 1 and 2

6.3.4.2 Effect of sire groups on measurements

There were no significant differences for production, efficiency and isotopes between the sire groups and parity had a large effect on measurements. NUE and milk $\delta^{15}N$ in the two periods were significantly different between parities ($P<0.001$ and $P<0.01$) in period 1, and NUE, milk $\delta^{15}N$ and $\Delta^{15}N$ were significantly different between parities (all $P<0.001$) in period 2.

6.3.5 Comparison of high and low breeding worth groups

Production, efficiency estimates, milk $\delta^{15}N$ and $\Delta^{15}N$ were significantly different between BW groups (Table 6.3). There was a significant linear decrease in LWT (kg) and DMI (kg/d) with increasing BW. There was a significant linear increase in NUE with increasing BW whilst milk $\delta^{15}N$ and $\Delta^{15}N$ linearly decreased with higher BW. Further correlation analysis showed BW was a weak predictor of NUE in periods 1 ($r = 0.20$; $P<0.01$) and 2 ($r = 0.20$; $P<0.001$). NUE was more related to PW ($r = 0.41$; $P<0.001$) and ($r = 0.32$; $P<0.001$) and LW ($r = 0.42$; $P<0.001$) and ($r = 0.34$; $P<0.001$) in periods 1 and 2 respectively. The differences in measurements between BW groups were numerically small, but still highly significant. Even with a 1% increase in NUE there can be economic costs saved through lower a higher milk solids at a lower intake and live weight.

Table 6.3 Mean production, efficiency and isotope data for animals grouped by BW in period 2 (n=100 in each group) and ANOVA comparison with associated s.e.d and P-values for BW group and polynomial responses (linear or quadratic).

	BW group					Difference		
	Low	Medium -Low	Medium	Medium -High	High	s.e.d[1]	*P*-value (BW group)	*P*-value (Linear response)	*P*-Value (Quadratic response)
BW, $	54	89	110	130	159	1.67	<0.001	<0.001	<0.01
PW, $	47	114	139	172	229	8.48	<0.001	<0.001	
LW, $	51	128	165	189	273	18.4	<0.001	<0.001	
LWT, kg	504	484	472	478	477	7.38	<0.001	<0.001	<0.01
DMI, kg/day	15.8	15.2	14.4	14.3	14.9	0.38	<0.001	<0.01	<0.01
Milk yield, kg/d	16.9	16.1	15.0	15.0	16.0	0.56	<0.01	<0.05	<0.01
Fat, %	6.72	6.65	6.58	6.48	6.37	0.14		<0.01	
Protein, %	4.29	4.36	4.47	4.50	4.49	0.05	<0.001	<0.001	
Lactose, %	5.14	5.15	5.19	5.19	5.18	0.02		<0.05	
Milk solids, kg/d	1.84	1.77	1.64	1.63	1.74	0.06	<0.01	<0.01	<0.01
Milk N, g/d	113	109	104	105	110	3.60	<0.05	<0.01	<0.01
Feed N, g/d	754	725	684	682	710	17.9	<0.001		<0.01
NUE, g/g	0.148	0.150	0.151	0.152	0.160	0.002	<0.01	<0.001	
MPE, g/g	0.369	0.363	0.357	0.351	0.347	0.003	<0.001	<0.001	
FCE, g/g	0.115	0.114	0.113	0.112	0.115	0.001			
Milk $\delta^{15}N$	7.16	7.10	7.07	7.03	6.93	0.06	<0.01	<0.001	
Milk $\Delta^{15}N$	0.74	0.67	0.65	0.61	0.51	0.06	<0.01	<0.001	

[1]s.e.d = Standard error of difference

6.4 Discussion

In this study there were only weak relationships between NUE and N isotope fractionation. This was likely because of the large excess of RDP from pasture that was not incorporated into microbial protein, which was not associated with N isotopic fractionation. There was preliminary evidence for between-animal variation in N partitioning related to ancestry and also differences in production, efficiency and isotope measurements for animals with varying BW.

6.4.1 Sample preparation

Sampling is important to consider when measuring N isotope fractionation because $\delta^{15}N$ is a sensitive technique. Previous evidence has suggested that using dried, ground samples of milk is the best method of preparation (Cheng, 2012); however this is more time consuming than using liquid samples. In this study there was a strong correlation between milk $\delta^{15}N$ from am and pm milk which is consistent with the strong correlation in milk $\delta^{15}N$ collected from am and pm samples in dairy cows in Cheng et al. (2010) ($r^2 = 0.74$; $P<0.001$). These results suggest that there are only small daily changes in milk $\delta^{15}N$. There were highly significant correlations between milk $\delta^{15}N$ that was prepared by two methods for IRMS which suggests that IRMS is not affected by the milk fat fraction that mixes with the rest of the milk sample when heating. Using fresh or chilled milk samples may be a better alternative when preparing large numbers of samples as it is less time consuming.

6.4.2 Production and dietary supply

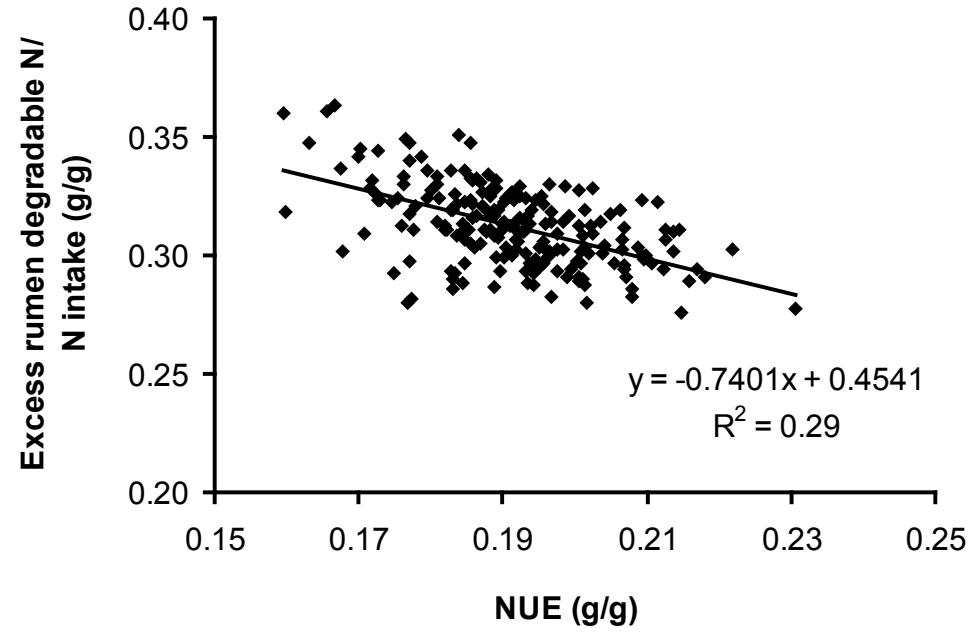
Pasture CP was high in both periods in this study (25.6 and 29.8 % in periods 1 and 2 respectively), which resulted in a large excess of N not required for microbial protein synthesis by the animal. Cows were more efficient and had higher milk yield in period 1 compared to period 2 whilst consuming similar intakes, reflecting the higher dilution of maintenance costs for increased milk output and the increased efficiency of the mammary gland at capturing amino acids for protein synthesis. Live weight

was lower in period 1 because earlier in lactation there is an increase in the utilisation of adipose tissue stores to support production (Bell et al., 2000; Friggens et al., 2004) because of a feed deficit which causes animals to be in negative energy balance.

The dominant factor determining NUE is N intake, which is likely to be highly correlated to RDP supply, so NUE is related to both factors. Therefore, it is difficult to say to what extent the differences in NUE were attributable to differences in RDP intake versus N intake. Both RDP and CP supply will be high from New Zealand pastures, so are both responsible for causing and excess of N that needs to be excreted as urea.

Metabolisable Protein intake (g/d) was approximately half of CP intake (g/d) (mean 49% MP) therefore NUE (g/g) was approximately half of MPE (g/g). Rumen degradable protein and microbial protein synthesis were calculated using equations by AFRC (1992). Rumen degradable-N (g/d) was 30-40% above requirements for microbial protein synthesis, and this excess would contribute to increased urinary N. Figure 6.3 (a) and (b) shows that lower levels of excess RDP were associated with more N efficient animals in both periods. Extrapolating to implied efficiency of N-use with zero excess RDP (g/g) in these figures shows high NUE in the absence of excess RDP (Figure (a) NUE = 0.61 g/g and (b) NUE = 0.58 g/g). This compares to the factor of conversion of metabolisable protein available for milk protein as 0.67 (NRC, 2001), 0.68 (AFRC, 1993) and 0.60 (Lapierre et al., 2005) in the literature. MP is used efficiently provided that it is close to the requirement of the animal and that there is enough energy to the animal.

(a)



(b)

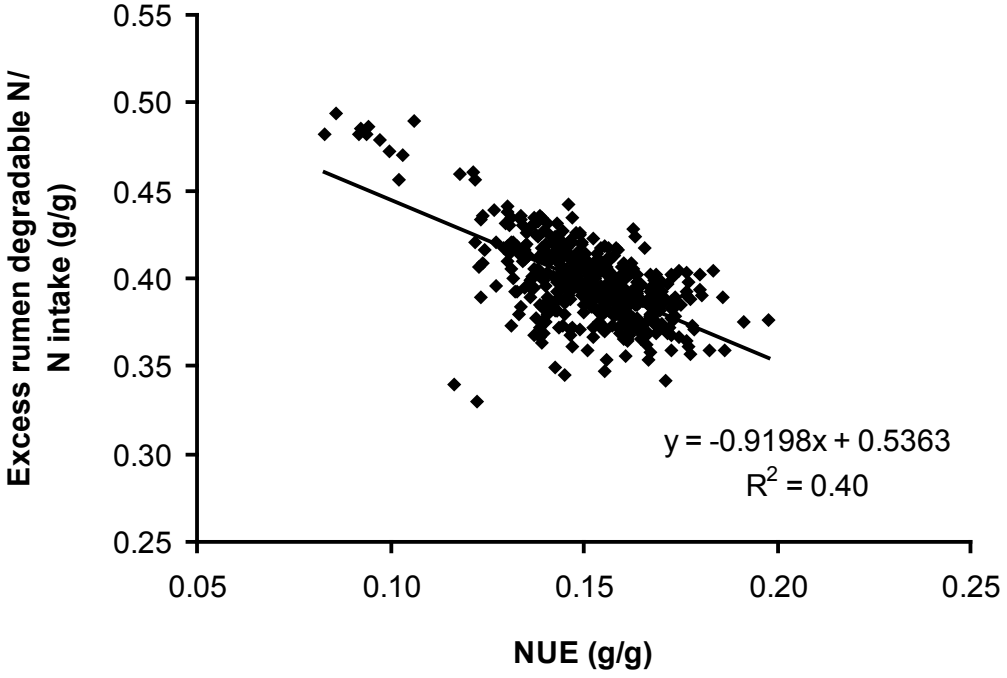


Figure 6.3 The relationship between NUE (g/g) and excess rumen degradable-N (expressed as RDP/N intake) for (a) period 1 and (b) period 2.

6.4.3 Relationship between NUE and N isotopic fractionation

There were weak relationships between NUE and milk $\delta^{15}N$ in this study and very low enrichment of milk $\delta^{15}N$ relative to the diet (average $\Delta^{15}N = 0.64$; s.d. = 0.44) in comparison with previous research with lactating cows ($\Delta^{15}N = 2.37$; Sutoh et al., 1987; $\Delta^{15}N = 2.68$; Cheng et al., 2011) and growing beef cattle ($\Delta^{15}N = 4.2$; Steele and Daniel, 1978; $\Delta^{15}N= 4.0$; Sponheimer et al., 2003). This was initially surprising given the low NUE, but suggests that $\Delta^{15}N$ may be driven by efficiency in the animal tissues, whilst NUE was driven by rumen efficiency. The weak relationship between NUE and N isotope fractionation may be related to apparent efficiency related to the use of absorbed amino acids for synthesis of body protein. This is supported by the implied high N-use efficiency in tissues with zero excess RDN (g/g) in Figure 6.3. $\Delta^{15}N$ was an indicator of the genetic variation in animal efficiency, but was not strongly related to NUE because it does not describe the effects of excess RDP on NUE. Urea synthesis is due to the catabolism of amino acids absorbed in excess of requirement, or excess ammonia absorption (both due to higher N intakes), both of which cause higher tissue enrichment of $\delta^{15}N$ relative to the diet. The increase in RDP increases milk protein, but also increases urinary N. The increase of milk protein reaches its maximum at 12.3% RDP, at which point any excess over is increasing urinary N and decreasing NUE and causing increased environmental effects (Reynolds et al., 2005).

6.4.4 Genetic variation between sire groups

Milk yield, milk protein and DMI were positively correlated between periods 1 and 2 because production and intake did not change (Table 6.2). There was a weak (but significant) correlation for NUE between periods 1 and 2 and there was no significant relationship for milk $\delta^{15}N$. All daughters were on the same diet but there may have been differences in N intake that affected NUE. The weak relationship also suggests there may have been differences in N utilisation (as a result of higher producing animals and/or lower N intake) compared to others, suggesting that NUE is not a consistent trait across lactation. The lack of relationship for NUE between

periods is mainly due to the change in dietary composition, in particular a 4.2% increase in CP which lowered NUE in period 2. The contribution of body reserves of the daughters, in particular fat reserves for milk synthesis may have also changed over the lactation.

There was no significant difference between sire groups in production, efficiency and N isotope fractionation once parity was taken into account. The range in NUE between sire groups was small (1.3%), therefore identifying animal-variation in this group of animals was difficult. Further analysis is required with a more complex model and more animals to determine differences in groups of animals with different sires.

6.4.5 Comparison of high and low breeding worth groups

The results in this study used estimations of intake and efficiency and include assumptions of ME requirements which make it difficult to reach conclusions. However, previous correlations between estimates of DMI and NUE with measured DMI and NUE (r = 0.71 and r = 0.86 respectively; Wheadon et al., 2012b) have provided some evidence that these estimates are relatively accurate. There are several systems to estimate DMI which are based on many different physiological factors and measurements (NRC, 1985), so the relationships in this study need further investigation before drawing conclusions on one example system. In addition, degradability of CP in the rumen can vary and information is limited. Chaves et al. (2006) found that the soluble CP fraction of perennial ryegrass mature leaves ranged from 35 to 61 %, and effective rumen degradable protein (ERDP) ranged from 49 (g kg^{-1} DM) for stem to 124 (g kg^{-1} DM) for leaf. These assumptions also affect the MPE values calculated in this study.

Based on the assumption that animals in the experiment were in zero energy balance (both periods in mid-lactation), high BW animals had lower estimated DMI and N intakes which may be a result of the lower live weight for high BW cows observed in the present study. There was no difference in milk yield for high BW cows compared

to low BW cows, which is likely due to the restriction of feed whereby high BW cows were unable to reach their maximum intake (Allen, 2000), so unable to consume sufficient energy for higher milk production. Previous findings by Woodward et al. (2011) noted high BW animals had higher DMI and N intakes in an indoor study, where feed may not have been restricted. However, the results still suggest that high BW cows have a higher genetic potential for feed utilisation for the production of milk protein at a lower feed intake (Dewhurst et al., 2002). This means that feed requirements of high BW animals would be lower due to their lower maintenance requirements (lower metabolic body weight) and also have higher gram per gram conversion of feed to milk protein than low BW cows.

There was no difference in FCE (milk solids kg/DMI kg) between high and low BW groups (Table 6.3), suggesting that specific N partitioning differences (i.e. NUE) may occur between animals of differing genetic merit independently of effects on FCE. BW is more related to processes underlying NUE than measures not directly concerning protein (e.g. fat yield) as protein has a high economic value in the BW calculation (BW = (Protein × NZ$8.63) + (Milk fat × NZ$1.79) + (Milk Volume × NZ$-0.09) + (LWT × NZ$-1.52) + (Fertility × NZ$7.35)+ (Residual Survival × NZ$0.15) + (Somatic cell × NZ$-38.57) (New Zealand Animal Evaluation Limited (NZAEL)). It is interesting to note that the most marked effect of BW on milk production was the large increase in milk protein % with increasing BW.

The higher estimated efficiency of high BW cows than low BW cows is in agreement with findings from Woodward et al. (2011) who used N balance techniques. The difference between high and low BW groups for NUE was small (1-2%) therefore there is difficulty in assessing the difference in metabolic efficiency between groups when CP levels were high. Cows offered lower protein diets (15.2% CP on a DM basis) showed a larger difference (4%) in N efficiency between high and low BW groups (mean NUE = 22 and 18.5% for high and low groups respectively) (Woodward et al., 2011), due to there being less excess RDP in the diet. The range in average BW ($) was similar in this study (54 to 159 for lowest and highest BW groups) to Woodward et al. (2011) (57 to 198 for low and high BW groups), however

N intake and milk N output was 8% and 28% higher in high BW cows compared to low BW cows in Woodward et al. (2011) compared to 6% and 3% lower in the present study due to their lower DMI compared to low BW cows and because cows had higher milk production (so higher NUE) in Woodward et al. (2011), and would have excreted less N in the urine.

There was a significant positive linear relationship between BW and NUE for the average of animals grouped by breeding worth which may be useful for predicting and comparing average N efficiency of groups of animals that are genetically similar. Analyses with individual cows showed that BW was a weaker predictor of NUE which is in contrast to the findings from Wheadon et al. (2012b) who found a significant positive relationship between BW and nitrogen use efficiency (r^2= 0.60) using measured intakes. The weak relationship between BW and NUE observed in this study may result from the weightings of other traits in the BW index, some (e.g. longevity, fertility) of which do not relate to components of efficiency. However, the results do show that animals with higher BW have higher NUE and partition more nutrients to milk which is in agreement with Davey et al. (1983). This variation in performance is due to the genetic differences in nutrient partitioning, but also regulation of protein metabolism pathways (McPherron and Lee, 1997). During lactation, higher BW cows may have increased ability to partition nutrients to the mammary gland and increase lipogenesis (Bauman and Currie, 1980). Older research by Trigg and Parr (1981) showed a lack of relationship between genetic merit and ME efficiency, further implying there are specific N partitioning differences.

6.5 Conclusions

There were weak relationships between NUE and N isotope fractionation in this study. The weakness of the relationship was likely caused by NUE being driven by a large excess of RDP that was not incorporated into microbial protein; whether the urea synthesis was due to the catabolism of amino acids absorbed in excess of requirements, or excess ammonia absorption (both due to higher N intakes), tissue enrichment of $\delta^{15}N$ would be higher relative to the diet. High BW cows have a higher genetic potential for increased feed utilisation but BW was a weak direct predictor of efficiency. Selection for cows with high BW may indirectly increase feed efficiency; however this process would be slow because of the weighting of other index traits. Further analysis is required with a more complex model and more animals to determine differences in groups of animals with different sires. Excess dietary RDP appears to be a limitation in the use of isotope fractionation for assessing NUE, therefore further grazing studies are required that have less RDP in the diet to investigate this theory. There was also a very low milk fractionation enrichment in this study, and limited pasture samples which suggests caution in the interpretation of these results.

CHAPTER 7

The relationship between nitrogen isotopic fractionation and nitrogen-use efficiency in parent and progeny pairs of dairy cattle

7.1 Introduction

Nitrogen loss from dairy cattle is a major contributor to environmental pollutants in the agriculture sector (Bleken et al., 2005). A large contributing factor to low NUE on dairy farms is exceptionally high amounts of protein in pasture (Watson and Atkinson, 1999). Increasing N intake linearly increases milk N, but also increases urinary N (Bockmann et al., 1997; Kebreab et al., 2001), but the slope for milk N is lower compared to urine (Dibb, 2000; Roberts, 2008). A major proposed solution for reducing emissions is genetic selection for improved feed efficiency (Crews jr, 2005), however there has been limited success due to the complexity of this trait (Ngwerume and Mao, 1992). Breeding strategies and indices need to integrate efficiency traits with others that are already selected for (e.g. longevity and milk yield) in order to be successful.

Using NUE as a measurement of feed efficiency in dairy cows may be a useful alternative strategy compared to other energy based measures of efficiency because it is less affected by changes in body composition during lactation (Wheadon et al., 2012a). For breeders to be able to select for NUE, simple and cost effective methods are required to measure this parameter in large groups of animals. Progress has been limited (Zamani et al., 2011) because there are difficulties and high costs associated with recording intakes for long periods of time (e.g. Herd et al., 2003). Consequently, there has been considerable interest in developing markers or proxies for feed efficiency. One example is MUN that is produced as a by-product of protein synthesis inefficiency (Stoop et al., 2007). Milk urea N indicates cows that have excess N in the diet (Nousianien et al., 2004; Stoop et al., 2007).

In this work we have focused on a new approach based on N isotope fractionation as a potential indicator of NUE. This method has been extensively used to understand N partitioning in archaeological and ecological research (Macko et al., 1986). N fractionation occurs when two naturally occurring isotopes such as ^{14}N and ^{15}N, differ in mass so behave differently in metabolic processes. The resulting fractionation (termed $\Delta^{15}N$) can be measured in biological samples (Kendall and Caldwell, 1998). Animal issues are more enriched (termed $\Delta^{15}N$; body tissue $\delta^{15}N$ – diet $\delta^{15}N$) than their diet (average 3‰ units; Sponheimer et al., 2003) because of the preferential utilisation of ^{14}N as it is lighter in mass (Minagawa and Wada, 1984; Macko et al., 1986; Macko et al., 1987; Sutoh et al., 1987). N fractionation has been a useful tool to measure N partitioning in non-ruminants e.g. plasma protein from rats (Sick et al., 1997), and hair protein from horses (Sponheimer et al., 2003) and in ruminants e.g. hair protein from cattle and goats (Sponheimer et al., 2003), $\Delta^{15}N$ as an indicator of NUE in Holstein-Friesian cows (Cheng et al., 2010; Cheng et al., 2011).

Potential indicators of feed efficiency (and NUE) must be heritable and repeatable in order to be used in selection; however there is often difficulty in gathering adequate data for heritability analysis (Bormann and Wilson, 2010). Estimates of heritability for new phenotypes can show individual differences in underlying biological processes (Visscher et al., 2008) and it is important to obtain estimates of genetic correlations to understand the response of the phenotype to selection (Lande and Price, 1989).

Heritability of feed efficiency has been estimated at between 0.07 (milk protein yield/ CP intake) and 0.40 (CP intake - milk protein yield) in dairy cows (Zamani et al., 2011) and has ranged from 0.16 to 0.43 in beef cattle (measured as RFI) (Herd et al., 2003), however there has been low phenotypic correlations with production and intake traits (Zamani et al., 2011).

The objective of this study was to investigate the relationship between N isotope fractionation and NUE in dairy cows. We also sought to assess heritability of NUE and $\delta^{15}N$ using a parent-progeny model.

7.2 Materials and methods

7.2.1 Measurement period

Seventeen mother and daughter pairs of Holstein-Friesian and Holstein-Friesian × Jersey cows were selected from the Lincoln University Research Dairy Farm (Lincoln, Canterbury, New Zealand), based on confirmed parentage from DNA testing (GeneMark DNA profile, Livestock Improvement Corporation). Animals (in their pairs) were randomly assigned to 3 groups (n=12, n=12, n=10) to accommodate animals in the experimental area over the course of 6 weeks. Cows were between 2 and 10 years of age and on average 168 (s.d = 21) days in milk at the start of the experiment; they were lactating and in calf. The experiment was approved by the Lincoln University Animal Ethics Committee (Application no. 509) under the Animal Welfare Act 1999, section 100 for the use of animals for research.

Prior to the experiment, each group was trained for 7 days to eat from individual feed bins in an indoor feeding area with a head gate. To encourage feed consumption, animals were held on a standoff area from morning milking to 1200h when they were offered up to 6kg/DM/cow grass silage for a 2h period, and then returned to pasture. Immediately after the training period, daily intake was individually recorded for 5 days (day 1-5). Animals were held on a standoff area between am milking and pm milking to encourage feed consumption; after feeding they were returned to the standoff until am milking.

Pasture was harvested from four 0.5ha paddocks that had been grazed previously, with N fertilizer applied at 120kg urea N/ha. The pasture consisted of perennial ryegrass with a small proportion of white clover (less than 15%) and paddocks were cut using a tractor with a forage harvester rotationally in strips at 1200h each day.

The number of strips harvested was based on pasture growth and previous daily intake. Cut herbage was collected and weighed into feeding bins (approximately 50kg fresh weight) and cows were offered free access to herbage *ad lib* after the pm milking (1400h) for 5 hours. Additional herbage was offered and weighed as required.

7.2.2 Sampling

Two samples were taken daily from each batch of cut herbage; one was freeze dried and prepared for compositional analysis (NIRS 5000/6500, FOSS Electric, Denmark), the other was used to determine DM content (oven dried for 48hrs at 40^{o}C). Biological samples were taken on days 1, 3 and 5 of each experimental period; milk samples were collected at am and pm milking and sent for analysis of fat, protein and lactose (Livestock Improvement Corporation, Hornby, New Zealand) or prepared for MUN analysis. Urine samples were collected mid-stream after vulval stimulation on days 1, 3 and 5 immediately after the feeding period and sub-sampled for total N, urea, ammonia, creatinine and purine derivative (day 5 only) analysis. Faeces were collected directly from the rectum after urine sampling. Sub-samples of faeces were used to determine DM content (oven dried for 36hrs at 100 ^{o}C) or freeze dried and ground to determine ash content and total N percent (NIRS, FOSS electric, Denmark). Blood samples were collected from the coccygeal vein using one vacutainer (9mL EDTA-coated vacuette, Griener Bio One) after feeding and before urine and faecal sampling on day 5 only. Subsamples of blood were used to prepare plasma; blood was spun at 3000g for 15 minutes at 4^{o}C to separate the plasma and was subsequently analysed for urea N.

In addition, subsamples of milk (am and pm), urine (days 1, 3 and 5), plasma (day 5) and herbage (day 1-5) were freeze dried and prepared for isotope ratio mass spectrometry (IRMS) for the analysis of the ratio of ^{14}N to ^{15}N, with 10% of samples analysed in duplicate (feed samples were all analysed in duplicate) (Iso-Trace Research, University of Otago, New Zealand).

Live weight was recorded after am and pm milking using an automated weigh scale. Am and pm milk yield were recorded using an automated system. Body Condition Score (BCS) (Roche et al., 2004) was taken on day 1 for each group. Animal information including date of birth, breed, BW, PW and genetic information including sire, dam and respective breeds, were obtained from the farm database.

7.2.3 Statistical Analysis

There were no experimental treatment groups in this study, with the main statistical focus to identify relationships between measured variables. The sample size of 15 pairs of animals was decided based on power tests which would provide a 90% statistical power of detecting a correlation when r = 0.7 (i.e. r^2 of approx. 50%) and also a 90% power of detecting 10% variation if attempting to identify systematic differences in traits between mothers and daughters using a paired t-test. The sample size chosen (34) also allows for drop out of individual animals (which by default would then lose data for the pair).

Preliminary analysis showed that there was no significant change in measurements over sample days; therefore for subsequent regression analyses an average of sampling days 1, 3 and 5 for each cow was used. Initial ANOVA showed a large group (or period; experiments spread over 6 weeks) effect, therefore this was used as a factor in linear regression analyses (Genstat 13th Ed; VSN International, 2010), which were used to analyse the relationships between NUE and milk, plasma, urine and herbage $\delta^{15}N$, as well as other metabolites.

Analysis of mother and daughter relationships also used the average of values from sample days 1, 3 and 5. Estimations of correlation coefficients between mothers and daughters were derived from REML linear mixed models (Kruuk, 2004) using daughter as the response variate, mother as the fixed model, and trial group, parity (age) and percent Jersey of the sire as random factors. According to Mendelian rules, inheritance between mothers and daughters should be equivalent (Lande and Price,

1989). Coefficients were used to estimate heritability using twice the slope of the regression line from the mixed model (Lynch and Walsh, 1998).

7.3 Results

7.3.1 Production traits and efficiency

All animals were successfully trained to use the feed intake system before the experiment. However, during the experiments 4 cows did not produce milk due to the restriction of feed intake which likely made the cows agitated and therefore did not stimulate the let-down reflex of milk flow. These cows were therefore excluded from further analyses.

The chemical composition and $\delta^{15}N$ content of herbage over the course of the experiment is summarised in Table 7.1. Hot and dry weather conditions contributed to a high range in CP (15.1 to 28.4 % of DM) and DM (13.4 to 20.2 %) in particular during weeks 3 to 5 of the experiment.

Table 7.1 Chemical composition, digestibility characteristics (% of DM unless otherwise stated) and $\delta^{15}N$ (‰) for pasture collected on each sample day (days 1 to 5) for each trial group.

Group	1 (n =12)					2 (n =12)					3 (n =10)				
Day of Trial	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
DM, %	15.1	21.8	19.4	17.1	24.3	20.7	28.0	28.4	22.6	27.5	24.8	21.1	23.7	20.7	17.4
OM	91.0	91.7	92.1	90.9	91.2	91.5	92.2	92.7	93.2	93.3	92.6	91.6	91.5	91.6	92.3
NDF	38.3	41.6	40.8	40.3	36.8	39.8	39.6	41.1	40.5	41.4	42.5	41.4	42.5	40.8	44.2
ADF	22.3	24.2	23.1	23.7	21.7	23.5	23.2	23.2	22.5	23.5	23.5	23.8	23.9	23.3	25.4
WSC	18.3	18.6	21.2	15.6	18.0	15.8	20.3	22.6	24.9	24.9	22.0	17.9	17.1	18.5	17.1
CP	18.5	16.5	16.0	18.2	20.2	18.1	15.7	15.0	14.0	13.4	15.3	17.3	17.6	17.8	16.7
DMD	79.3	77.9	79.2	78.2	80.6	76.9	78.0	79.0	80.8	80.0	78.4	76.4	76.6	77.5	75.2
OMD	84.5	82.7	84.4	83.1	86.2	81.5	83.0	84.0	86.1	85.0	83.4	81.1	81.6	82.6	79.8
DOMD	77.1	75.7	77.5	75.6	78.5	74.7	76.6	77.9	80.4	79.4	76.9	73.9	74.1	75.2	73.2
$\delta^{15}N$, ‰	2.87		2.70		2.16	2.26		2.91		2.57	2.20		1.93		1.62

There was a significant change in DMI over the course of each experiment ($P<0.001$) and there were significant differences between groups ($P<0.001$; Figure 7.1). Changes in DMI did not correlate to changes in herbage $\delta^{15}N$ or changes in diet composition e.g. CP.

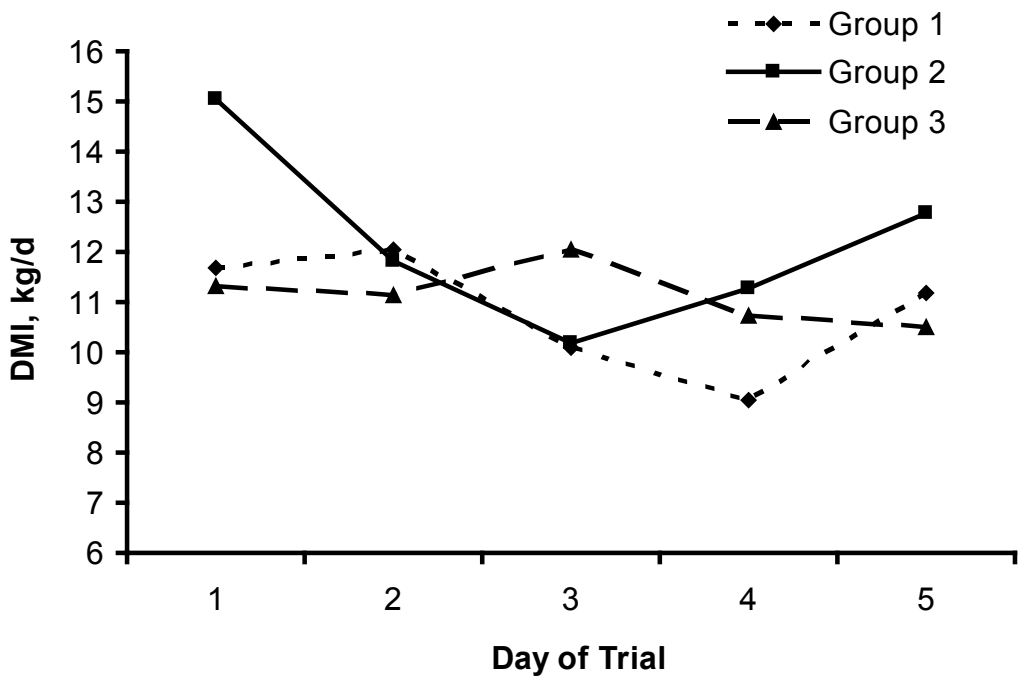


Figure 7.1 Changes in dry matter intake (kg/d) over the course of the experiment (days 1 to 5) for trial group 1 (n=12), 2 (n=12) and 3 (n=10) (pooled s.e.m = 0.49).

Average milk production and composition data for all animals is summarised in Table 7.2. There was no significant change in LWT or milk yield over the course of the experiment. NUE was positively correlated with milk solids production (kg/d) (r = 0.57; P<0.001) and milk yield (kg/d) (r = 0.58; P<0.001).

Table 7.2 Mean BW, production, milk composition and efficiency data averaged for individual animals for sample days 1, 3 and 5 and averaged for all trial groups (n=34).

	Mean	s.d.
BW[1], $	74	44
PW[2], $	61	102
BCS	4.04	0.46
LWT, kg	438	81
DMI, kg/d	11.6	2.1
N intake, g/d	288	81
Milk yield, kg/d	13.1	2.8
Protein, %	3.65	0.41
Fat, %	5.74	0.93
Lactose, %	4.88	0.48
Protein, g/d	483	91.8
Fat, g/d	745	169
Lactose, g/d	648	142
Milk Solids[5], kg/d	1.23	0.25
Milk N, g/d	75.8	14.4
NUE[3], g/g	0.29	0.09
FCE[4], g/g	0.11	0.03

[1]Breeding worth
[2]Production worth
[3]Nitrogen-use efficiency (g milk N/ g feed N)
[4]Feed conversion efficiency (milk solids kg/d / DMI kg/d)
[5]Protein (g/d) + Fat (g/d)

7.3.2 Isotope measurements

Mean herbage, milk, plasma and urine $\delta^{15}N$ were 2.40 (s.d. = 0.30), 5.33 (s.d. = 0.24), 5.17 (s.d = 0.32) and -1.36 (s.d = 0.34). Mean $\Delta^{15}N$ milk and $\Delta^{15}N$ plasma were 2.93 (s.d = 0.27) and 2.77 (s.d = 0.44), with an enrichment range between 1.82 to 3.62 and 2.49 to 3.53 units for milk and plasma respectively. There were significant differences among groups for milk $\delta^{15}N$ ($P<0.01$), plasma $\delta^{15}N$ ($P<0.1$), $\Delta^{15}N$ milk ($P<0.001$) and $\Delta^{15}N$ plasma ($P<0.001$) (Table 7.3). There was also individual variation within groups with some cows maintaining higher milk $\delta^{15}N$ values over time than others.

Table 7.3 Mean $\delta^{15}N$ (‰) milk, plasma and urine and $\Delta^{15}N$ (plasma $\delta^{15}N$ ─ herbage $\delta^{15}N$ or milk $\delta^{15}N$ ─ herbage $\delta^{15}N$) and s.d for each group (including mothers and daughters).

Group	$\delta^{15}N$ Milk	SD	$\Delta^{15}N$ Milk	SD	$\delta^{15}N$ Urine	SD	$\delta^{15}N$ Plasma*	$\Delta^{15}N$ Plasma*
1	5.14	0.16	2.89	0.39	-1.30	0.33	5.03	2.46
2	5.09	0.14	2.75	0.21	-1.47	0.40	5.34	2.76
3	5.02	0.18	3.20	0.25	-1.32	0.42	5.15	3.24

*Day 5 only

There were significant correlations between am and pm milk $\delta^{15}N$ in samples taken on the same day (r = 0.69 to 0.84). There were also significant correlations for milk $\delta^{15}N$ between sample days; there was a strong correlation between days 3 and 5 and a weaker correlation between days 1 and 3 and days 1 and 5. In addition, there were significant group differences ($P<0.001$; Table 7.4). There were weaker correlations between days 1, 3 and 5 for urine $\delta^{15}N$; the correlation was particularly weak between days 1 and 3 (r = 0.23; n.s.) compared to days 3 and 5 (r = 0.62; $P<0.001$) and between days 1 and 3 (r = 0.53; $P<0.01$) and there were no significant differences between groups.

Table 7.4 Correlation matrix for the relationship between milk $\delta^{15}N$ (weighted on milk yield for am and pm) on sample days 1, 3 and 5 for trial group (a) 1, (b) 2 and (c) 3. Significance levels are ***P<0.001, **P<0.01, *P<0.05, †P<0.1.

(a)

Day 1	-		
Day 3	0.76**	-	
Day 5	0.51†	0.86***	-
	Day 1	Day 3	Day 5

(b)

Day 1	-		
Day 3	0.80**	-	
Day 5	0.65*	0.76*	-
	Day 1	Day 3	Day 5

(c)

Day 1	-		
Day 3	0.64†	-	
Day 5	0.81*	0.88**	-
	Day 1	Day 3	Day 5

There was a moderate correlation between milk $\delta^{15}N$ and plasma $\delta^{15}N$ ($P<0.01$; Figure 7.2) on day 5, for both am ($r = 0.46$; $P<0.01$) and pm ($r = 0.50$; $P<0.01$) milk samples.

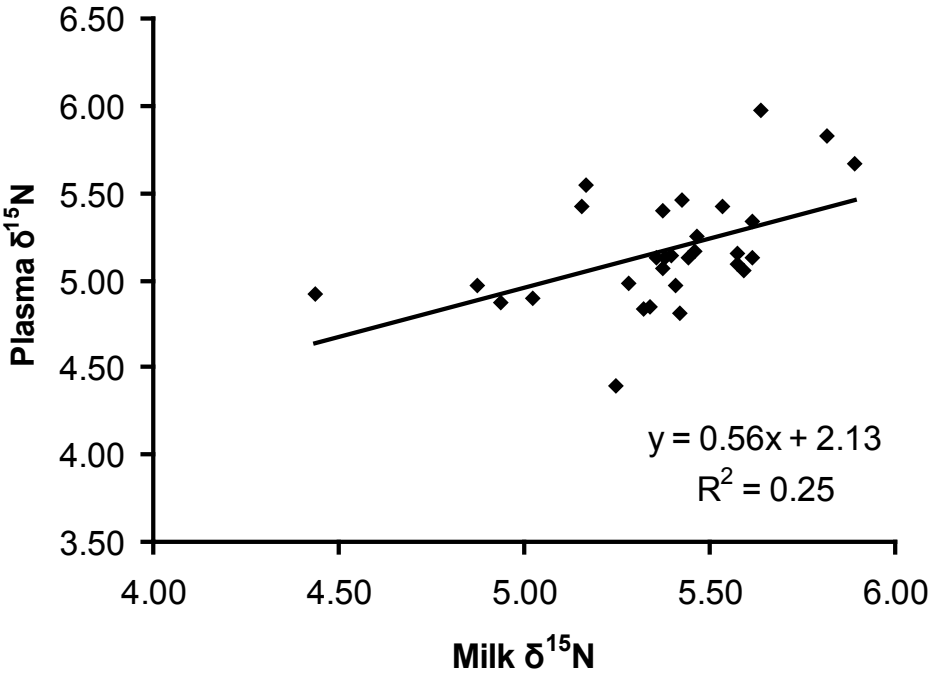


Figure 7.2 The relationship between milk $\delta^{15}N$ (‰) (weighted daily milk $\delta^{15}N$ using am and pm milk yield (kg/d)) and plasma $\delta^{15}N$ (‰) for all trial groups averaged for sample days 1, 3 and 5 for each animal (n=34).

There was a significant negative relationship between NUE and plasma $\delta^{15}N$ (r^2=0.23; P<0.01) and $\Delta^{15}N$ plasma (P<0.001; Figure 7.3). There was also a significant negative relationship between NUE and plasma $\delta^{15}N$ and $\Delta^{15}N$ plasma when group was added as a factor in the regression analysis (P<0.05) and (P<0.05) respectively. There was no significant relationship between NUE and milk $\delta^{15}N$ or $\Delta^{15}N$ milk, however there was a weak negative relationship between NUE and $\Delta^{15}N$ milk when group was added as a factor in the regression analysis (P<0.1). There was no significant relationship between NUE and urine $\delta^{15}N$.

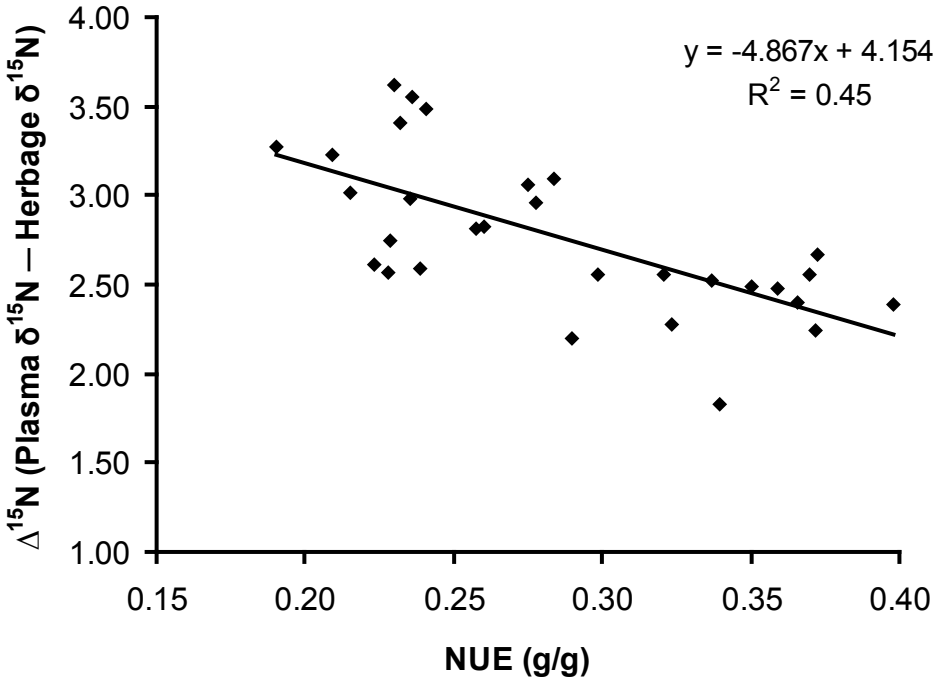


Figure 7.3 The relationship between N-use efficiency (milk N g/d/ feed N g/d) and $\Delta^{15}N$ plasma (plasma $\delta^{15}N$ (‰) ─ herbage $\delta^{15}N$ (‰) for all trial groups averaged for sample days 1, 3 and 5 for each cow (n=34).

7.3.3 Relationship between NUE and other metabolites

A summary of mean milk, urine, faeces and blood metabolites are shown in Table 7.5. There was no significant relationship between NUE and MUN (mmol/l) and blood urea N (BUN) within groups. There were no significant relationships between NUE and any urine analytes within groups apart from a negative relationship with uric acid (mmol/l) ($P<0.05$).

Table 7.5 Mean (s.d.) values for the composition of plasma, milk, urine and faeces

	Mean	s.d.
Plasma, mmol/l		
Urea N	11.1	2.81
Milk, mmol/l		
MUN	6.93	1.55
Urea	3.46	0.77
Urine concentrations		
N, %	0.59	0.09
NH3, mmol/l	4.00	2.54
Creatinine, mmol/l	2.66	0.54
Urea, mmol/l	147	28.6
Purine Derivatives, mmol/l		
Allantoin	5.68	1.54
Hypoxanthine	0.058	0.036
Xanthine	0.022	0.021
Hippuric acid	18.5	7.66
Uric acid	0.56	0.18
Faeces, %		
DM	21.36	0.24
Ash	20.02	2.17
N	2.89	0.20

7.3.4 Mother and daughter relationships

Production and composition data for the comparison of mothers and daughters is shown in Table 7.6. Mothers had higher LWT and DMI, as well as milk yield and milk solids compared to their daughters (confirmed by paired t-test; all $P<0.001$). There was no significant difference in efficiencies, blood, urine and faeces metabolites, or isotope measurements between mothers and daughters, but there were significant differences between mother-daughter pairs.

Table 7.6 ANOVA comparison and associated s.e.d and P-value of milk production and composition, feed intake and isotope analysis for mother and daughters.

	Mothers		Daughters			
	Mean	s.e.	Mean	s.e.	s.e.d	P-value
BCS	4.10	0.12	3.93	0.10	0.15	NS
LWT, kg	494	7.04	386	9.24	11.61	P<0.001
Milk yield, kg/d	14.7	0.41	11.7	0.24	0.47	P<0.001
DMI, kg/d	12.7	0.23	10.8	0.29	0.37	P<0.001
N intake, g/d	315	10.3	268	10.9	15.0	P<0.05
Milk solids, kg/d	1.36	0.04	1.10	0.02	0.05	P<0.001
Fat, g/d	827	26.4	662	15.0	30.4	P<0.001
Protein, g/d	534	14.26	435	9.57	17.2	P<0.001
Lactose, g/d	713	22.6	584	13.9	26.5	P<0.001
Milk N, g/d	83.7	2.24	68.1	1.50	2.69	P<0.001
NUE, g/g	0.282	0.013	0.279	0.014	0.019	n.s
FCE, g/g	0.109	0.004	0.106	0.004	0.005	n.s
BW $	53	5.08	98	4.96	7.10	P<0.001
PW $	42	15.0	80	11.8	19.1	n.s
δ^{15}N Milk	5.31	0.04	5.26	0.05	0.06	n.s
δ^{15}N Plasma	5.17	0.08	5.13	0.08	0.11	n.s
δ^{15}N Urine	-1.31	0.07	-1.23	0.07	0.10	n.s
δ^{15}N Herbage	2.39	0.06	2.38	0.06	0.08	n.s
Δ^{15}N Plasma	3.02	0.10	2.98	0.12	0.16	n.s
Δ^{15}N Milk	2.92	0.05	2.88	0.06	0.08	n.s

There were significant correlations between mothers and daughters for some production and efficiency traits, summarised in Table 7.7. Heritability estimates were very high using the slope coefficients from the linear mixed models. Estimates for milk yield (kg/d), BUN (mmol/d), NUE (g/g), milk $\Delta^{15}N$ and plasma $\Delta^{15}N$ were 0.60 (s.e = 0.14), 1.28 (s.e = 0.15), 0.54 (s.e = 0.18), 1.60 (0.37) and 1.42 (s.e = 0.19) respectively for models with random factor trial group and 0.68 (s.e = 0.16), 1.32 (s.e = 0.16), 0.64 (s.e = 0.17), 0.98 (s.e = 0.22) and 1.82 (s.e =. 0.16) for models with random factors trial group, parity and sire Jersey percent.

Table 7.7 Regression coefficients from REML mixed models (and associated significance, F-prob) for the relationship between mothers and daughters for efficiency, isotope and metabolite measurements. Model structure is daughter as the response variate, mother as fitted model, plus random factors: trial group, parity and sire Jersey breed percent.

(a) Including random factors: Trial group

x	Equation	F-prob
Milk yield, kg/d	7.21 (s.e. = 2.06) + 0.30x (s.e. = 0.14)	0.051
Lactose yield, kg/d	332 (s.e. = 125) + 0.34x (s.e. = 0.17)	0.067
BUN, mmol./l	3.60 (s.e. = 1.74) + 0.64x (s.e. = 0.15)	0.011
NUE, g/g	0.19 (s.e. = 0.05) + 0.27x (s.e. = 0.18)	n.s
Plasma $\delta^{15}N$, ‰	1.94 (s.e. = 1.06) + 0.63x (s.e. = 0.20)	0.011
$\Delta^{15}N$ Milk	0.55 (s.e. = 1.10) + 0.80x (s.e. = 0.37)	n.s
$\Delta^{15}N$ Plasma	0.86 (s.e. = 0.55) + 0.71x (s.e. = 0.19)	0.004

(b) Including random factors: trial group, parity and sire Jersey %

x	Equation	F-prob
Milk yield, kg/d	6.97 (s.e. = 2.10) + 0.32x (s.e. = 0.14)	0.045*
Lactose yield, kg/d	312 (s.e. = 127) + 0.37x (s.e. = 0.17)	0.053*
BUN, mmol./l	3.22 (s.e. = 1.78) + 0.67x (s.e. = 0.16)	0.005
NUE, g/g	0.18 (s.e. = 0.05) + 0.32x (s.e. = 0.17)	0.093
Plasma $\delta^{15}N$, ‰	1.48 (s.e. = 0.94) + 0.72x (s.e. = 0.18)	<0.001
$\Delta^{15}N$ Milk	0.64 (s.e. = 1.05) + 0.77x (s.e. = 0.35)	0.048*
$\Delta^{15}N$ Plasma	0.31 (s.e. = 0.44) + 0.90x (s.e. = 0.16)	<0.001

*Random factors trial group and sire Jersey % only.

7.4 Discussion

There is a demand for cost effective methods to measure feed efficiency in large groups of animals for inclusion in breeding programs. In this experiment, N isotopic fractionation, measured in milk or plasma, was related to NUE. There were production, efficiency and isotope differences between groups and pairs of animals and there were high correlations and heritabilites for some production and efficiency traits between mothers and daughters.

7.4.1 Production traits and efficiency

Average DMI was 11.6 kg/d in this study, which is higher than estimates based on daily ME requirements for maintenance, lactation and activity (Nicol and Brookes, 2007). The short time period allocated for recording herbage intake resulted in the restriction of daily intake; despite being offered herbage *ad lib*, the animals could not consume their daily volume in the time period allocated due to rumen fill, which is a limitation of this approach. There was a range in individual DMI, from 8.7 to 14.0 kg/d (Mean =11.6; s.d. = 2.1). Average NUE was 29% and ranged up to 50%, which is similar, and somewhat higher than recent estimates of efficiency (Sutton et al., 2013). There was a positive correlation between milk yield and NUE reflecting the dilution of maintenance costs for increased milk output. NUE was also increased by a reduction in N intake. The adaptation to the reduction in intake and subsequent changes in N metabolism allowed cows to maintain a steady milk yield throughout the experiment.

7.4.2 Relationship between N isotopic fractionation and NUE

In this experiment, NUE was negatively related to (plasma) $\delta^{15}N$ and (plasma) $\Delta^{15}N$ which is consistent with findings from Cheng et al. (2010) using milk from dairy cows supplemented with urea. It is also consistent with the negative relationship between plasma $\Delta^{15}N$ and FCE in the study by Wheadon et al. (2014) with growing beef heifers. Enrichment ($\Delta^{15}N$) of plasma and milk averaged 2.77 and 2.93 respectively in this study, which is slightly higher than in other studies using lactating animals (e.g. $\Delta^{15}N = 2.59$, Koyama et al., 1985; $\Delta^{15}N = 2.37$, Sutoh et al., 1987; $\Delta^{15}N = 2.68$, Cheng et al., 2010) but lower than studies using growing beef animals (e.g. $\Delta^{15}N = 4.2$, Steele and Daniel, 1978; $\Delta^{15}N = 4.0$, Sponheimer et al., 2003). Higher enrichment can be explained by the restriction of feed which made animals more N efficient so decreased the extent of isotopic fractionation. Urine isotope data was consistent with the enrichment of animal tissues in $\delta^{15}N$ compared to the diet, which leaves urine depleted in $\delta^{15}N$ (Sick et al., 1997). This is a result of the combination of urea synthesis from amino acids and microbial degradation of protein to ammonia (Wattiaux and Reed, 1995), which increases urine N excretion pathways. The mammary gland is efficient at the capture of amino acids for protein synthesis so dairy cows use MP efficiently, and waste little to urine.

Milk $\delta^{15}N$ was correlated between am and pm and over time in each experiment. There were weaker correlations between day 1 and other days because animals had only just been introduced to the feed intake recording regime. The largest change in milk $\delta^{15}N$ and urine $\delta^{15}N$ was between day 1 and 3 because N metabolism was affected by the initial reduction in intake. There were stronger correlations between days 3 and 5 because animals had adjusted their metabolism and adapted to the reduction in intake.

There was a moderate correlation between milk $\delta^{15}N$ and plasma $\delta^{15}N$ (Figure 7.2), despite the fact milk was measured before and plasma after feeding. Plasma $\delta^{15}N$ is likely to be more stable over time because of the slower rate of turnover of protein in plasma (Waterlow, 1984; Boldt, 2010). Therefore there was no significant

relationship between NUE and milk $\delta^{15}N$ or Δ^{15N} milk because milk protein is synthesised and secreted more rapidly. Plasma $\delta^{15}N$ is therefore a more robust measurement as it fluctuates less over time and is more representative of NUE over a longer period of time.

Plasma N isotopic fractionation was a relatively good indicator of NUE in this study, and explained more variation in NUE than blood metabolites and hormones previously used to predict feed efficiency in ruminants (e.g. Nousianien et al., 2004; Richardson et al., 2004; Kelly et al., 2010; Lawrence et al., 2012). The variation not explained by this relationship is likely the result of the fluctuation in herbage $\delta^{15}N$ over time, and the feed restriction which resulted in physiological changes when animals entered the experiment.

7.4.3 Relationship between NUE and other metabolites

Blood urea N (mmol/l) was greater (mean 11.06; s.d. = 2.81) than MUN (mmol/l) (mean 6.93; s.d. = 1.55) in this study which is consistent with other literature (Broderick et al., 1997) but they were less correlated than relationships previously observed (mean r^2 = 0.36). Milk urea nitrogen has been used to indicate the rumen efflux of crude protein to show N losses of rumen fermentation, but does not represent the efficiency with which absorbed protein is utilised (Hof et al., 1997). Blood urea N measurements were from 1 sample after feeding, compared to 6 MUN values (am and pm days 1, 3 and 5) so MUN was more representative of urea concentrations over time. A strong correlation between MUN and blood urea N has also been reported, with both MUN and blood urea N being sensitive to changes in the supply of CP, RDP and RUP, but insensitive to the changes in amino acid balance (Roseler et al., 1993; Baker et al., 1995). The half-life of urea is shorter compared to plasma protein (Regoeczi et al., 1964) suggesting N fractionation in plasma is a more robust method than MUN or BUN.

Uric acid represented 4-10% of total purine derivatives excreted in the urine, similar to findings in Giesecke et al. (1994) and Linberg et al. (1991). There was a negative

relationship between NUE and uric acid which is in contrast to previous research that has showed an improvement of rumen microbial efficiency with increased purine derivatives (Dijkstra et al., 2013); however in some cases they are not accurate indicators of microbial N flow (Johnson et al., 1998). This may be a result of changes in physiological state that caused alterations in recycling of purines into nucleic acids, changes in excretion routes, changes in the amount of purine losses (Johnson et al., 1998).

7.4.4 Relationship between mothers and daughters

Although pairs of animals were randomly allocated to groups, they differed in production and efficiency during the experiment e.g. group 1 had higher NUE and lower intake than group 2 and 3. There were also differences in milk yield between groups during the 7 days before the experiment (mean 16.0, 13.6 and 14.1 kg/d for groups 1, 2 and 3 respectively). Changes in production may be related to changes in pasture quality (Table 7.1) and suggests that there was between-animal variation in NUE reflected in the different groups. This is consistent with previous evidence that energy and protein metabolism can differ between animals of varying genetic merit (Woodward et al., 2011; Wheadon et al., 2013). The experiment was also over the course of 6 weeks, therefore group 3 may have had different production requirements compared to group 1 due to the stage of lactation changes.

7.4.5 Heritability estimates

In this study estimates of heritability were unrealistically high for some traits, which suggest that using the parent-offspring regression method overestimated heritabilities. Although the sample size was based on power calculations, the experiment may have been underpowered which may have biased the results. The inflation of heritability may have been caused by common or maternal environment effects, which cause increases in the covariance between mothers and daughters (Kruuk, 2004). Other sources of random bias effects include maternal and adaptive maternal effects (both of which have a genetic and environmental component)

(Muller et al., 2012), permanent environmental effects, genotype-environment interactions (which can affect expression of covariance) (Kruuk, 2004) and effects caused by selection and inbreeding, or migration and mutations of alleles from new variants (Visscher et al., 2008). A trait with high heritability may still be heavily influenced by environment; therefore differences between populations are not necessarily due to differences in genotype (Vleck and Bradford, 1965). Designing regression models to predict daughter outcomes from mothers would need to include a number of factors to account for systematic differences between groups of animals on experimentation at different times (Visscher et al., 2008).

Preliminary results in this study suggest that efficiency traits and N fractionation have a genetic component. There were significant correlations between mother and daughters for many efficiency and production traits, and blood, urine and faeces metabolites suggesting that some of these traits may heritable. There were also significant differences in production, efficiency and N isotopes for different pairs of mothers and daughters suggesting potential to identify parents with higher efficiency in breeding programmes which are likely to pass down desirable traits to their daughters. However, due to large group effects, the over estimation of heritability estimates in this study suggests the need for a larger genetic study using an animal model with large numbers of animals rather than the mother-daughter model. Environmental conditions were constant in this study but more complex analyses are required to account for other bias factors such as maternal effects which if not taken into account can lead to mis-leading heritability estimates.

7.5 Conclusion

N isotope fractionation in plasma was a moderately good predictor of NUE, which may be a useful tool to predict feed efficiency in dairy cattle breeding programs under conditions of restricted intake with herbage of moderate CP content. The variation not explained by the relationship was in part a result of changes in diet $\delta^{15}N$ and adaptation to the restriction of feed intake. Plasma was more robust than milk because plasma protein has a longer half life and so fluctuates less over time. The depletion of urine was consistent with the enrichment of animal tissues in $\delta^{15}N$ compared to the diet. There was no relationship between NUE and both BUN and MUN, and also no relationship between NUE and the urine metabolites measured apart from uric acid. There were differences between groups and pairs in several measurements suggesting there is genetic variation between animals, but the relationship of N utilisation and isotope measurements between mothers and daughters and associated heritability was not conclusive and requires further study with a larger sample.

CHAPTER 8

GENERAL DISCUSSION

8.1 Introduction

Feed efficiency is an important area of research in cattle, particularly the identification of animals that are divergent for nutrient utilisation. There is a large amount of research about nutritional effects on energy and N partitioning in ruminants, but less work on the underlying genetics of these traits. Research in this thesis was centred on the identification of between-animal variation in groups of animals that were offered the same diet, rather than investigating the effects of diet differences.

Biochemical markers that predict feed efficiency phenotypes would have great potential to be used where diet composition is unknown or where feed intake cannot be recorded. This would accelerate the collection of feed efficiency data in large numbers of breeding stock and further advance the rate of genetic selection for feed efficient animals. Literature searches showed that previous research has not identified reliable metabolic predictors or proxies for feed efficiency or components of feed efficiency. In contrast, the worked reported in this thesis has identified potential for using N isotopic fractionation as an indicator of feed efficiency in growing beef cattle and free grazing dairy cows.

The main objectives of this thesis were to (i) evaluate the reliability, advantages and disadvantages of using N-use efficiency (NUE) as a measurement for feed efficiency in dairy cows, in comparison with other energy-based measures, (ii) investigate the relationship between N isotopic fractionation and feed efficiency in beef, and (iii) dairy cattle (in particular in free grazing dairy cows), and (iv), understand genetic factors that affect these relationships (e.g. sire effects, heritabilities between parents and progeny). Long term application of this information would be in investigating the underlying genes that are associated with N utilisation; however it is important to

provide both confidence and repeatability in the marker before considering a genome-wide approach. This also included understanding NUE and N isotope fractionation through generations and its heritability. The genome wide approach would be beneficial in understanding the underlying genetic control of N utilisation in the future; however in the short term, using phenotype markers for feed efficiency in combination with breeding strategies is faster and less costly than identifying gene regions associated with NUE.

Experiments in this thesis involved groups of cattle; beef heifers in chapter 4 and lactating dairy cows in chapter 3 and 5 to 7. These cattle were in different herds and locations, with a large range in feed efficiency and production traits. There were repeatable significant negative relationships between feed efficiency (in particular FCE) and N isotopic fractionation. Variation in this relationship (in particular for dairy cows) was explained by nutritional (e.g. protein supply and quality; feed intake), environmental (e.g. pasture growth) and genetic effects (e.g. sire and breed). This discussion highlights the potential for N isotopic fractionation to be used as a biomarker for feed efficiency and the main factors that contribute to variation in this relationship. Preliminary evidence of genetic effects will be discussed, ending with conclusions and recommendations for future research.

8.2 Expressions of feed efficiency

In this thesis, feed efficiency was either described as ECE (MJ milk energy/MJ metabolisble energy intake) (Chapter 3), FCE (g live-weight gain/ g DM intake) (Chapter 4), or NUE (g milk N/g feed N) (Chapter 5-7). A summary of mean feed efficiency in each Chapter, using these different metrics, is summarised in Table 8.1.

Table 8.1 Summary of animal information (physiological state; mean days in milk) and feed efficiency (with s.d. and range) for each experiment.

Animal Description	Location	Physiological state	*n*	Days in Milk	Measurement	Efficiency (g/g)	s.d.	Range
Dairy cows (Chapter 3)	UK	Lactating	38	Full Lactation	ECE[1]	0.36	0.06	0.19-0.38
Dairy cows (Chapter 3)	UK	Lactating	38	Full Lactation	NUE[2]	0.20	0.02	0.12-0.24
Beef heifers (Chapter 4)	Ireland	Growing	84		FCE[3]	0.09	0.03	0.04-0.15
Dairy cows (Chapter 5; Period 1)	Ireland	Lactating	135	151	NUE[2]	0.24	0.03	0.18-0.34
Dairy cows (Chapter 5; Period 2)	Ireland	Lactating	135	182	NUE[2]	0.20	0.02	0.15-0.25
Dairy cows (Chapter 6; Period 1)	New Zealand	Lactating	200	165	NUE[2]	0.19	0.01	0.16-0.23
Dairy cows (Chapter 6; Period 2)	New Zealand	Lactating	550	181	NUE[2]	0.15	0.02	0.08-0.20
Dairy cows (Chapter 7)	New Zealand	Lactating	34	168	NUE[2]	0.29	0.09	0.19-0.40

[1]ECE = MJ milk energy/MJ metabolisble energy intake
[2]NUE = g milk protein/ g N intake
[3]FCE = g live-weight gain/ g DM intake

The range in NUE reported (8 to 40%) is broader than ranges reported for lactating cows by Castillo et al. (2000) (15 to 35%) and lactating cows and goats in Cheng (2012) (16 to 25% and 11-15% respectively). Highest efficiency was associated with dairy cows that were feed restricted (Chapter 7). The range in NUE was lowest for the study reported in Chapter 6 (7 and 12% in periods 1 and 2 respectively) compared to animals in Chapter 7 (21%). NUE was higher for dairy cows in studies conducted in Ireland compared to those conducted in New Zealand, which was largely because of differences in CP content in the pasture. Studies were mainly conducted in mid-lactation, and there was an indication that NUE was higher in early lactation. Lowest efficiency was associated with growing beef heifers, which is because they are at a lower level of production than dairy cows (less 'dilution of maintenance'), whilst the efficiency of utilisation of absorbed amino acids is lower for protein accretion in muscle than for milk protein synthesis (AFRC, 1992; discussed further in 8.2.3).

8.2.1 Measuring feed efficiency in dairy cows

Feed efficiency estimates in dairy cows are more difficult to interpret because of the mobilisation and replenishment of body reserves that occurs during lactation and late gestation (Madhav et al., 1997; Friggens et al., 2004; Prendiville et al., 2009; Williams et al., 2013). In chapter 3, NUE was investigated for its suitability for describing feed efficiency in dairy cows. NUE was highly correlated to an energy-based measure of efficiency (ECE) because they are both driven by the effects of dilution of maintenance costs for energy and protein as well as pathways for synthesis of milk components (Vandehaar and St-Pierre, 2006; Khan et al., 2013). There were small differences in the relationship that were driven by differences in metabolic efficiency of maintenance processes (Baldwin, 1968; Baldwin, 1987). The higher variation in ECE, especially during early lactation, made NUE a more stable target than ECE for use in dairy cows.

8.2.2 Efficiency of utilisation of amino acids for live weight gain and milk production

Dairy cows had higher NUE than the FCE measured in growing beef heifers in this thesis. Despite being different measurements, NUE is a component of FCE and the differences between results for beef and dairy cattle may be attributed to, or a combination of (i) quality and quantity of dietary protein supply, (ii) absolute maintenance requirements and (iii), dilution of maintenance costs for production and respective efficiency of gain (k_{ng}) or protein synthesis (k_{nl}) for beef and dairy cattle.

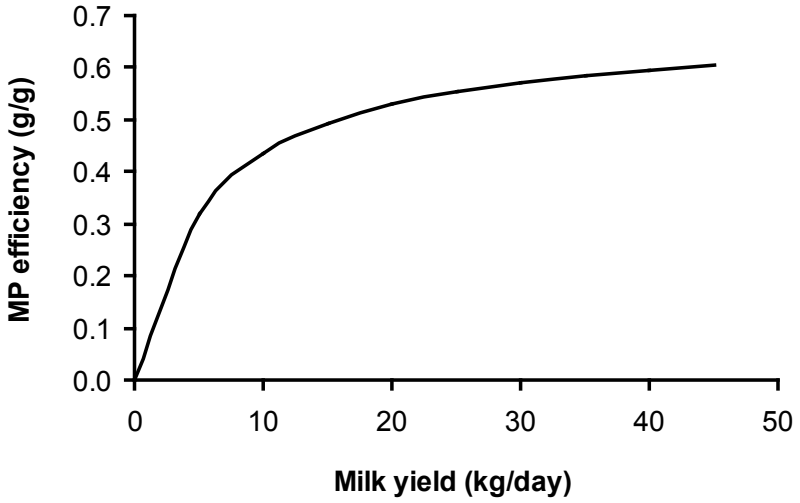
Absorbed amino acids originating from microbial protein or digestible UDP represents the total quantity of amino acids available for metabolism (MP or truly absorbed amino acids) (Figure 2.1; Chapter 2). Only 75% of the microbial protein is amino acids, and a further 85% is digestible, so 68% is metabolisable (AFRC, 1992). Efficiency of milk synthesis is high for dairy cows (83%; Baldwin, 1986; 81 to 84%; Onken et al., 2011) and the efficiency of utilisation of amino acids is greater for lactating animals (k_{nl} = 0.68) than growing animals (k_{ng} = 0.59) (AFRC, 1992).

Dairy cattle have a higher dilution of maintenance for milk production than beef cattle for LWT gain because of their higher production level (ARC, 1980; AFRC, 1992). Variation in dairy cattle maintenance requirements (at the same feed intake and milk production) can also account for up to 37.6% of the variation in milk energy efficiency (Onken et al., 2011). This variation is mainly driven by protein turnover, ion pumping and proton leakage which is a major function of maintenance requirements and accounts for 30 to 40% of basal energy expenditure (Baldwin, 1980).

The effects of production level (milk yield in dairy cattle; growth rate in beef cattle) on the efficiency of utilisation of amino acids was modelled using AFRC (1992) values (Figure 8.1 (a-b)). The dairy cow model assumed a 600kg dairy cow producing between 0 and 45 kg milk/day with 3.2% protein. The beef model was for a 300kg steer with growth rates from 0 to 1.5 kg/day and the protein content of LWT

gain from ARC (1980). The dilution of maintenance effect is evident in the increasing efficiency in Figure 8.1. A large part of the increase in efficiency due to 'dilution of maintenance' was achieved at relatively low production levels (less than 10 kg milk/day). Whilst this modelling showed lower efficiencies for beef cattle, the increase in efficiency owing to 'dilution of maintenance' is more evident across the normal range of productivity (i.e. up to 1.5 kg/day).

(a)



(b)

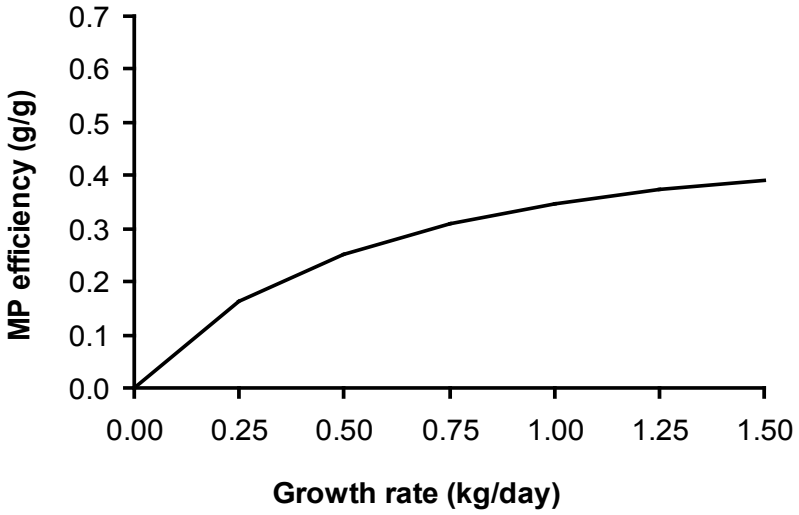


Figure 8.1 Simulated efficiency of utilisation of amino acids for (a) milk production (kg/d) for a 600kg dairy cow with 3.2% milk protein and (b), LWT gain (kg/d) for a 300kg steer (protein in LWT gain from ARC (1980)).

8.3 Nitrogen isotopic fractionation as a biomarker for feed efficiency

8.3.1 Nitrogen isotopic fractionation data

Nitrogen isotopic values were measured in plasma, milk, urine and pasture in the studies reported in this thesis. A summary of mean $\delta^{15}N$ and $\Delta^{15}N$ for samples collected in each experiment is shown in Table 8.2. There was a range in $\delta^{15}N$ in the diet, which was reflected by dietary composition, study location and grazing management. In addition, different sources of N may have contributed to intra-plant variation in $\delta^{15}N$ (discussed further in 8.4.1).

Plasma $\Delta^{15}N$ in growing beef heifers was slightly lower than previous studies using growing animals e.g. $\Delta^{15}N = 4.2$ (Steele and Daniel, 1978), $\Delta^{15}N = 4.0$ (Sponheimer et al., 2003). Most previous research involved mature animals that would have ceased growing and so use dietary N relatively inefficiently, resulting in a high level of isotopic fractionation ($\Delta^{15}N$). Lower $\Delta^{15}N$ has been reported in studies with suckling young and in most studies with lactating ruminants (Chapter 2; Table 2.3). It would be expected that these more productive animals would be using N more efficiently, partitioning a greater proportion of N to muscle or milk protein and less to urea.

Table 8.2 Summary N isotopic fractionation and $\Delta^{15}N$ for all experiments

Animal Description	*n*‡	Diet $\delta^{15}N$	s.e.m	Milk $\delta^{15}N$	s.e.m	Urine $\delta^{15}N$	s.e.m	Plasma $\delta^{15}N$	s.e.m	$\Delta^{15}N$	s.e.m
Beef heifers; Chapter 4	84	4.95	0.10					8.53	0.36	3.58	0.37
Dairy cows; Chapter 5, P1	135	3.62	0.50					7.55	0.50	3.92	0.50
Dairy cows; Chapter 5, P2	135	4.84	0.48					7.55	0.30	2.71	0.49
Dairy cows; Chapter 6, P1	200			7.23	0.50						
Dairy cows; Chapter 6, P2	550	6.42	0.00	7.06	0.44					0.64	0.44
Dairy cows; Chapter 7	34	2.38	0.31	5.28	0.27	-1.30	0.38	5.15	0.32	2.76 (Plasma)	0.43
										2.90 (Milk)	0.29

‡Number of animals in the study

There was a large range in plasma or milk $\Delta^{15}N$ in dairy cows (mean 0.64 to 3.92 units relative to the diet). Most studies were similar to previous research using lactating animals e.g. $\Delta^{15}N = 2.59$ (Koyama et al., 1985), $\Delta^{15}N = 2.37$ (Sutoh et al., 1987), $\Delta^{15}N = 2.68$ (Cheng et al., 2010), except for Chapter 6 which showed very low enrichment relative to the diet (linked with high pasture $\delta^{15}N$) and Chapter 5 (period 1) which showed higher levels of enrichment. Some of this discrepancy may be related to large variation between samples of pasture compared to plasma; there was a high rate of change in pasture $\delta^{15}N$ which varied between paddocks and time points in Chapter 5 and between trial days in Chapter 7. There may also have been differences in fractionation across studies that arose from bias due to analytical variation. Milk $\delta^{15}N$ also had a high rate of change (daily), in contrast to plasma which did not change over time (chapter 4, 5, 6; further discussed in section 8.3.3). Urine was depleted by approximately 1‰ relative to the diet, which is consistent with the enrichment of animal tissues in $\delta^{15}N$, and with other literature observations (Steele and Daniel, 1978; Sick et al., 1997).

8.3.2 Relationship between N isotope fractionation and feed efficiency

There were significant negative relationships between N isotope fractionation in plasma and feed efficiency in growing beef heifers (Chapter 4) and dairy cows (Chapter 7), which may be useful for application in cattle breeding, evaluating feed efficiency without measuring feed intake and diet composition (providing that animals are fed with identical diets), and be used to compare the nutrient use efficiency of different feeds. Regression equations for the relationship between N isotopic fractionation and feed efficiency in each study are summarised in Table 8.3. There were significant negative relationships between FCE and plasma $\delta^{15}N$ and $\Delta^{15}N$, repeated over 4 time points in beef heifers which were driven by the paritioning of N between liveweight gain and excretion in urine, and significant negative relationships between NUE and plasma $\delta^{15}N$ and $\Delta^{15}N$ in dairy cows (Figure 8.2; Figure 8.3). In contrast, studies using milk showed weaker or no significant relationships between NUE and $\delta^{15}N$ or $\Delta^{15}N$.

Table 8.3 Linear regression equations and significance for the relationship between N isotopic fractionation ($\Delta^{15}N$) and feed efficiency (g/g) in each experiment

Animal Description	*n*	Sample	Equation	r^2	*P*-Value
Beef heifers (Chapter 4)[1]	84	Plasma	$\Delta^{15}N = 4.22$ (s.e = 0.10) ─ 7.36 (s.e = 1.10) × FCE (g/g)	0.35	<0.001
Beef heifers (Chapter 4)[2]	20	Plasma	$\Delta^{15}N = 4.79$ (s.e =. 0.21) ─ 10.05 (s.e = 2.11) × FCE (g/g)	0.56	<0.001
Dairy Cows (Chapter 5; P1)[3]	135	Plasma	$\Delta^{15}N = 5.00$ (s.e = 0.33) ─ 2.16 (s.e = 1.41) × NUE (g/g)	<0.10	n.s
Dairy Cows (Chapter 5; P2)[3]	135	Plasma	$\Delta^{15}N = 3.25$ (s.e = 0.29) ─ 1.95 (s.e = 1.57) × NUE (g/g)	<0.10	n.s
Dairy cows (Chapter 6; P2)	550	Milk	$\Delta^{15}N = 2.35$ (s.e = 1.26) + 0.28 (0.19) × NUE (g/g)	0.10	<0.1
Dairy cows (Chapter 7)[4]	34	Plasma	$\Delta^{15}N = 4.11$ (s.e = 0.31) – 4.72 (s.e = 1.07) × NUE (g/g)	0.45	<0.001
Dairy cows (Chapter 7)[4]	34	Milk	$\Delta^{15}N = 3.28$ (s.e = 0.26) – 1.25 (s.e = 0.90) × NUE (g/g)	<0.10	n.s

[1]Whole population analysis using breed as a factor in the model
[2]Average of subset time points using breed as a factor in the model
[3]General linear model using treatment group (1 to 9) as a factor
[4]Using treatment group as a factor in the model

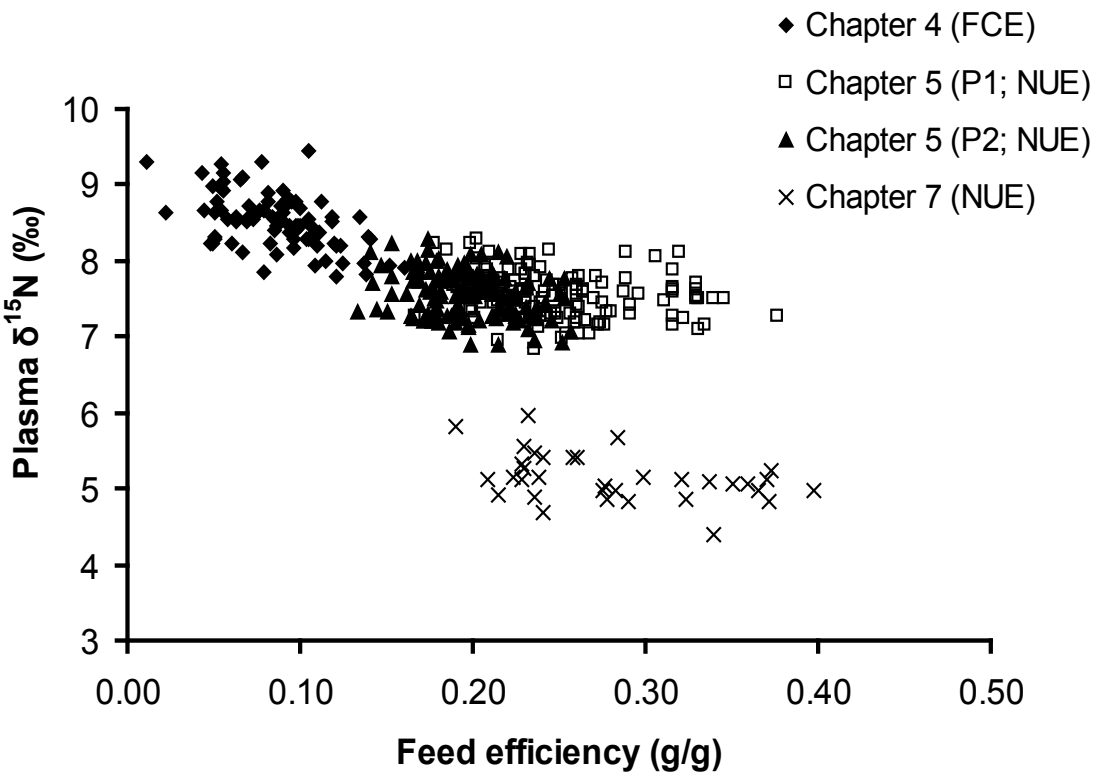


Figure 8.2 Relationship between feed efficiency (g/g), measured as either FCE (g live-weight gain/ g DM intake) or NUE (g milk N/ g feed N), and plasma $\delta^{15}N$ (‰).

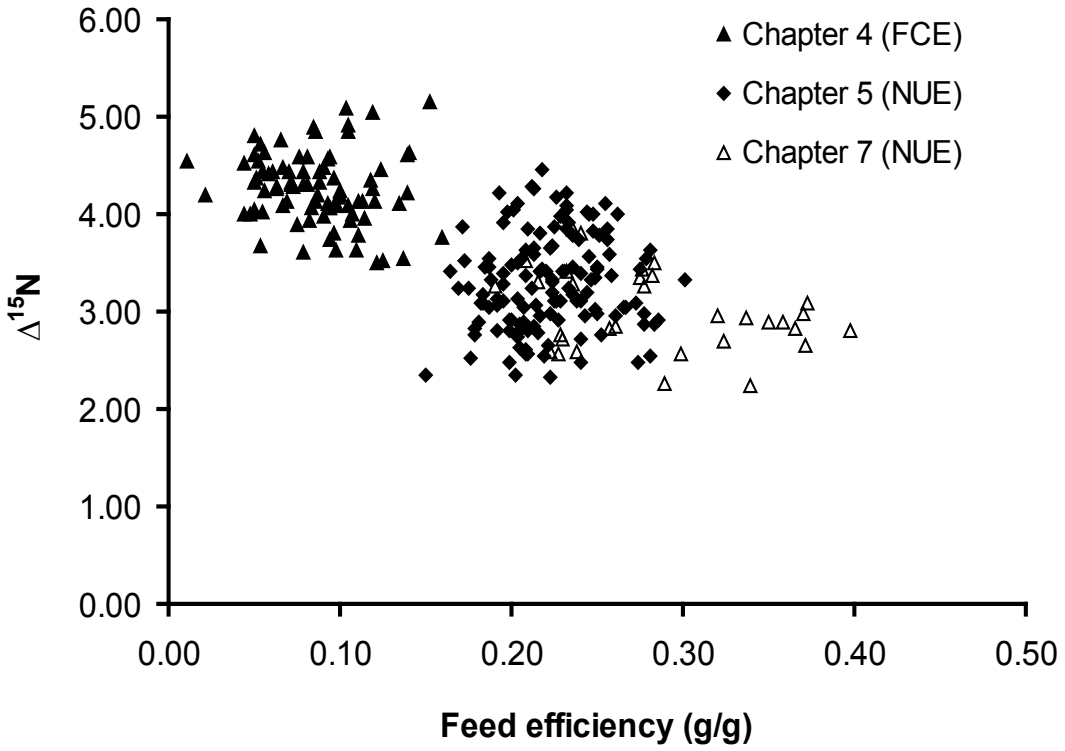


Figure 8.3 Relationship between feed efficiency (g/g), measured as either FCE (g live-weight gain/ g DM intake) or NUE (g milk N/ g feed N), and $\Delta^{15}N$ (Plasma $\delta^{15}N$ – diet $\delta^{15}N$).

A comparison of the relationships reported in this thesis with similar studies is summarised in Figure 8.4. Across a wide range of studies involving different species, physiological states and diets, there is a consistent negative relationship between the enrichment of animal tissues in $\delta^{15}N$ compared to the diet, with increasing feed efficiency. The studies in this thesis, with just one exception (Chapter 6) confirm the negative relationship shown in previous studies. It seems likely that the lack of relationship in Chapter 6 is related to the very high pasture CP which made apparent NUE very low (Table 8.2). It is likely that the efficiency of utilisation of amino acids for milk protein synthesis was high from MP but there was a large excess of RDP that was above animal requirements and so was excreted as urine (section 8.4.4). The negative relationship between $\Delta^{15}N$ and FCE is consistent with the increased $\Delta^{15}N$ when cattle and goats were fed diets containing higher protein levels (Sponheimer et al., 2003). In dairy cows, the negative relationship between plasma $\Delta^{15}N$ and NUE is consistent with findings from Cheng et al. (2010) using milk from dairy cows supplemented with urea. Cheng (2012) also reported significant negative relationships between muscle enrichment (muscle $\delta^{15}N$ – diet $\delta^{15}N$) and retained N/N intake in non lactating sheep, and milk and plasma $\Delta^{15}N$ and NUE in lactating cows.

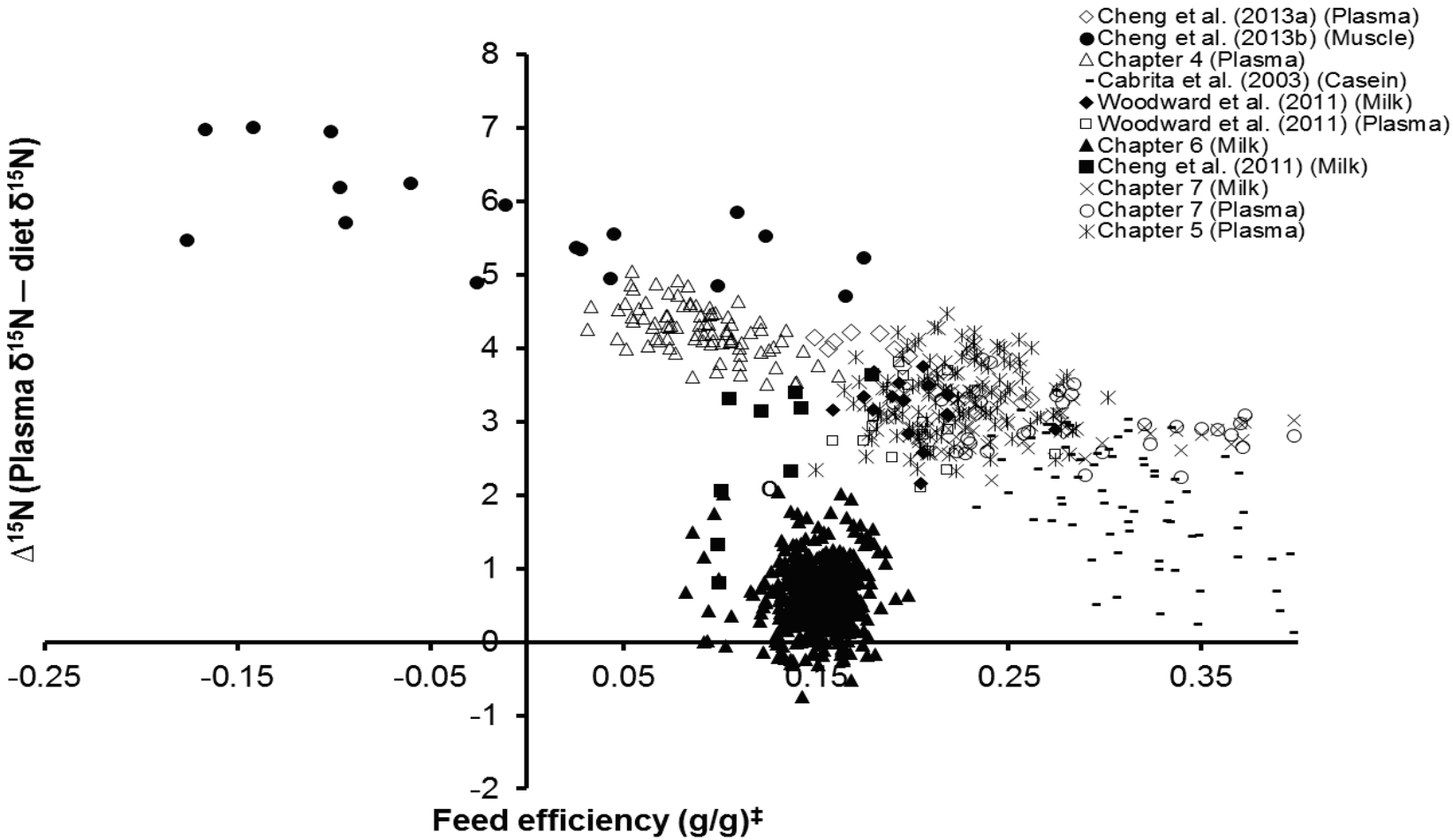


Figure 8.4 The relationship between N isotopic fractionation and feed efficiency (‡Measured as either FCE (g live-weight gain/ g DM intake) or NUE (g milk N/ g feed N) in this thesis and other literature.

Plasma $\Delta^{15}N$ explained more variation in NUE and FCE in animals fed the same diet (based on regression models) than blood metabolites and hormones that were used to predict feed efficiency in earlier studies (Chapter 2; Table 2.2). In this thesis, MUN was not a reliable indicator of protein inefficiency. There was no significant relationship between NUE and MUN in Chapter 7, which is in contrast to previous research that has found MUN to be useful indicator of protein metabolism e.g. Jonker et al., 1998; Nousianien et al., 2004. As pasture CP was closer to animal requirements in Chapter 7 compared to other studies, it unusual that MUN was not associated with NUE, however MUN was not measured in the other studies to compare.

In Chapter 6 a preliminary study was conducted to compare $\delta^{15}N$ in milk prepared for IRMS by two different methods, and samples collected at am and pm milking were also compared. There was a positive correlation between am and pm milk $\delta^{15}N$ which is consistent with the positive correlation between am and pm milk $\delta^{15}N$ reported by Cheng et al. (2010). These results suggest there were small changes in $\delta^{15}N$ within one sample day. However, the correlation was weaker between milk $\delta^{15}N$ sampled over several days and with greater time separation (Chapter 7). In contrast, plasma $\delta^{15}N$ was highly correlated over 4 time points in the study reported in Chapter 4. Plasma $\delta^{15}N$ may be a more stable measurement than milk $\delta^{15}N$ over time because of the slower rate of turnover of protein in plasma (Waterlow, 1984; Boldt, 2010) and therefore is more representative of NUE over a longer period of time. The relationships between NUE and milk $\delta^{15}N$ and $\Delta^{15}N$ were probably weaker than those based on plasma samples because milk protein fluctuates daily as it is synthesised and excreted rapidly. This means milk is more sensitive to changes in N isotopes in the diet (Hobson et al., 1993; Sponheimer et al., 2003), but it makes it less reliable as a marker over time compared to plasma. The half life of urea is also shorter compared to plasma protein (Regoeczi et al., 1964) suggesting that measuring N isotope fractionation in whole plasma or plasma protein is more robust than in milk urea N or plasma urea N.

8.4 Sources of variation in feed efficiency and N isotopic fractionation

8.4.1 Variation in diet composition and pasture $\delta^{15}N$

Despite the fact animals were on the same diet within each study, there was variation in pasture composition and pasture $\delta^{15}N$ collected at different time points and paddocks (including samples collected only one day apart). N isotopic fractionation in different tissues is generally expressed by subtracting the feed (Sutoh et al., 1993), however if there is high variation in pasture $\delta^{15}N$ over a short time period, using plasma $\delta^{15}N$ without subtracting the feed may be a more reliable indicator of the animal effects on N isotope fractionation. Location and climate were a main source of the variation in diet composition and consequently feed efficiency (Table 8.4).

Table 8.4 Summary diet composition and $\delta^{15}N$ data in each study, g/kg DM unless otherwise stated

Animal Description	Location	Diet	Diet Component						
			DM, %	CP	NDF	ADF	DMD*	ME† (MJ/kg DM)	$\delta^{15}N$ (‰)
Beef heifers; Chapter 4	Ireland	Grass silage	24.3	136	511		744[1]	10.7[3]	3.20
Beef heifers; Chapter 4	Ireland	Concentrate	8.6	140	215		858[1]	13.4[3]	5.74
Dairy cows; Chapter 5, P1	Ireland	Pasture	16.7	207			764[1]	11.9[3]	3.62
Dairy cows; Chapter 5, P2	Ireland	Pasture	15.7	224			771[1]	12.0[3]	4.84
Dairy cows; Chapter 6, P1	New Zealand	Pasture	19.4	256	450	245	795[2]	12.4[3]	
Dairy cows; Chapter 6, P2	New Zealand	Pasture	12.9	298	488	244	796[2]	12.4[3]	6.42
Dairy cows; Chapter 7	New Zealand	Pasture	22.2	167	401	234	783[1]	12.2[3]	2.38

*Dry matter digestibility; [1]measured *in vitro,* [2]estimated as DMD = DOMD divided by 0.98 + 4.8 (MAFF, 1984)

†Metabolisble Energy (MJ/kg DM) estimated as 0.016 × DOMD (g/kg DM) (× 0.0155 for grass silage) (MAFF, 1984)

Pasture had high CP content in studies conducted in New Zealand, particularly that reported in Chapter 6, and as a consequence diet $\delta^{15}N$ varied within and between experiments, and $\delta^{15}N$ was positively related to CP levels (r^2 for experiment means = 0.40) (Figure 8.5).

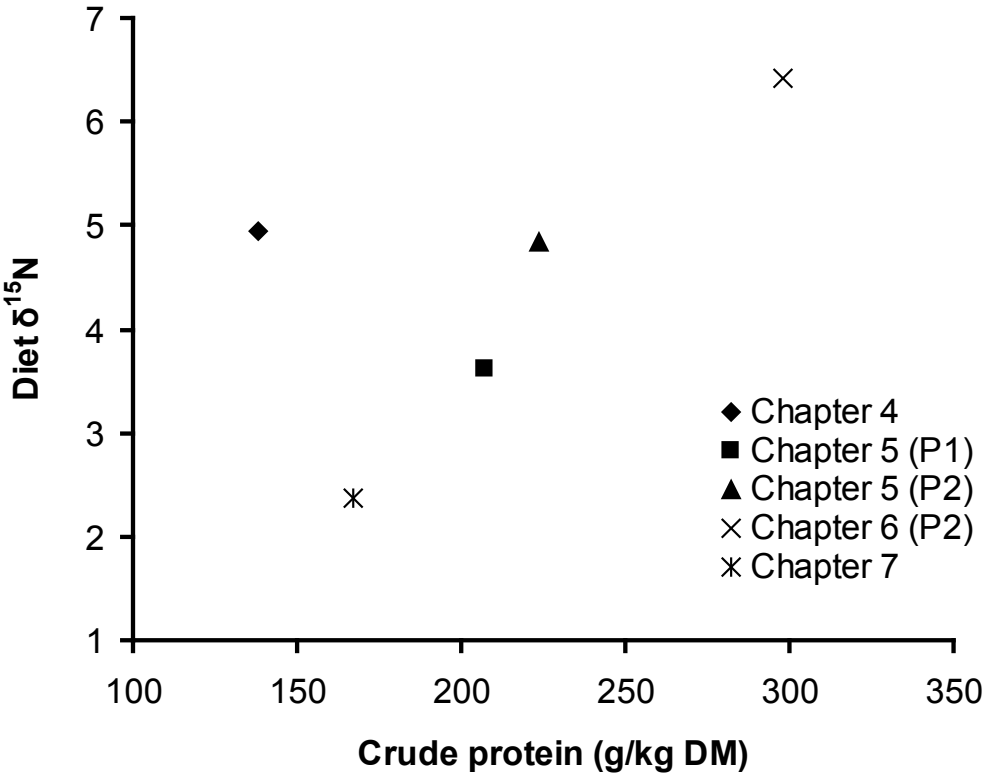


Figure 8.5 Relationship between crude protein (g/kg DM) and diet $\delta^{15}N$ for experiment means

Care must be taken when drawing conclusions on this relationship based on treatment means of only 5 studies, however previous research on plant physiology can help to explain this relationship. Intra-plant variation in $\delta^{15}N$ can occur through a number of factors e.g. organ-specific losses of N, differences in N assimilation and reallocation of N in the plant (Evans, 2001), and also may be caused by differences in the sources of N (e.g. NO_3^- and NH_4^+) which have a major effect on intra-plant variation in $\delta^{15}N$ (Yoneyama and Kaneko, 1989; Yoneyama et al., 1991). More variation occurs when NO_3^- is the primary source compared to NH_4^+ because there are differences in the pattern of assimilation (Evans, 2001). Differences in grazing management (e.g. rotational grazing movements) and N fertiliser regimes would also affect the source of N in the soil and consequently uptake of the plant organs which would affect the isotope signature of the sample when collected. Pasture growth depends on fixation of atmospheric N_2 by bacteria, oxidation of ammonia to nitrate (Delwiche and Steyn, 1970) and denitrification (Wellman et al. 1968), all of which have different isotopic fractionation effects (Rennie et al. 1976) which could have contributed to differences in pasture $\delta^{15}N$.

8.4.2 Excess dietary nitrogen

Partitioning of N (and N isotopic fractionation) between protein (muscle or milk) and urea is affected by protein supply and quality (Sick et al., 1997; Poupin et al., 2011). The differential metabolism of N isotopes is related to NUE and dietary N intake, through the absorption of ammonia or digestion of protein in the small intestine. In the case of increasing dietary N, an increasing proportion of N is directed to urine so the fractionation rate increases (causing a higher isotopic signature), because higher amounts of ammonia and urea are formed in the urea cycle. If there is more urea synthesis from catabolised amino acids (and potentially ammonia) there will be more enrichment of body tissues and depletion of urea. Therefore whether the urea synthesis is due to the catabolism of amino acids absorbed in excess of requirement, or excess ammonia absorption (both due to higher N intakes), tissue enrichment of $\delta^{15}N$ would be higher relative to the diet. Both RDP and CP supply will be high from

New Zealand pastures, so are both responsible for causing and excess of N that needs to be excreted as urea.

8.4.2.1 Excess rumen degradable protein

The dominant factor determining NUE is N intake, which is likely to be highly correlated to RDP supply, so NUE is related to both factors. Therefore, it is difficult to say to what extent the differences in NUE were attributable to differences in RDP intake versus N intake. The increase in RDP increases milk protein, but also increases urinary N. The increase of milk protein reaches its maximum at 12.3% RDP, at which point any excess over is increasing urinary N and decreasing NUE and causing increased environmental effects (Reynolds et al., 2005).

As discussed in 8.4.1, there was variation in CP which in most studies was higher than required by the animal. Calculations to estimate the excess RDP that was not required in the diet (RDP; g/d) in relation to N intake (g/d)) (AFRC, 1992) showed an excess of 19 to 29% (Chapter 5) and 30 to 40% (Chapter 6) RDP above requirements that was not incorporated into microbial protein and so was excreted as urinary N. Excess RDP was also associated with less N efficient animals in these studies because RDP is used less efficiently than MP as it cannot be used directly for protein synthesis.

Inefficiency in the use of dietary protein results both from inefficient conversion of RDP into microbial protein and inefficient use of absorbed amino acids (MP) for milk protein synthesis. RDP that is in excess of requirements for microbial protein synthesis is wasted, being largely excreted in the urine. MP supply is used efficiently for milk protein synthesis providing that the supply is close to requirements; if there is an excess of MP relative to energy supply, it will be as wasteful as the excess of RDP in the rumen. As most fractionation occurs in animal tissues (e.g. liver), lower urine N excretion from inefficiency of animal tissues compared to the rumen results in lower N isotopic fractionation effects. The proportion of urea recycled from dietary N is expected to be low when N intake is greater than requirements

(Reynolds and Kristensen, 2008) so the relationships between N isotopic fractionation and NUE were mainly driven by deamination or transamination in the liver tissues (Macko et al., 1986; Parker et al., 1995). This suggests that $\Delta^{15}N$ may be driven by efficiency in the animal tissues, whilst NUE was driven by rumen efficiency particularly when cows were offered high protein herbage.

Studies with the highest excess RDP showed only weak relationships between N isotopic fractionation and feed efficiency, because whilst $\Delta^{15}N$ may be an indicator of the genetic variation in animal efficiency, the relationship may have been diluted by the effects of excess RDP on NUE. At lower levels of CP, closer to requirements (Chapter 7), N isotope fractionation was able to detect variation in overall N efficiency. The dilution of maintenance effect is largest for mid- to high-yielding cows, but there is little change in predicted NUE driven by tissue-level effects at these levels of yield (Figure 8.1), hence there is little change in N isotopic fractionation and only weak relationships. If there is a constant excess of RDP, there is no increase extra increase of milk production and no extra N to microbial protein from amino acids, so no change in $\delta^{15}N$ absorbed for milk sinks (Cheng, 2012).

RDP is a major contributor to the poor relationships between isotope fractionation and NUE, so conducting a complex genetic trial wold need to be assessed with a small (or zero) excess of RDP in the diet, however measuring efficiency under these conditions would not demonstrate typical New Zealand conditions. Testing either a low CP diet or diet based on more RUP products e.g. canola meal, corn and soybean to bypass rumen fermentation may have potential. As the efficiency of MP is high for milk production, there should be a high efficiency and low isotope fractionation with these feeds, however this could also cause problems for rumen fermentation and there may be increased recycling of N from microbes which could cause a fractionation effect.

8.4.3 Genetic factors

Variation in the composition of pasture (and grass silage in chapter 4) and excess RDP contributed to the variation in feed efficiency and N isotope fractionation and their relationship in this thesis. However, since in most cases all animals received the same diet in each study (so the same excess N and RDP, or the same excess RDP and N within a group), there is preliminary evidence to suggest that changes in N partitioning were the result of the genetic variation in feed utilisation. The animal variation in N efficiency in each study is consistent with Davey et al. (1983), who also showed variability in the conversion of nutrients to milk with animals offered same diet and there is evidence that that energy and protein metabolism can differ between animals of varying genetic merit (Woodward et al., 2011).

8.4.3.1 Sire effects and heritability

Sire groups were formed in the study reported in Chapter 6 in order to compare efficiency and production in groups of daughters. There were no significant differences between sire groups because sire differences were strongly associated with parity, and the range in NUE between sire groups was very small (1.3%). Cows in the herd were also likely to be closely related, so identifying animal-variation in related groups was difficult using simple statistical analysis. Heritability of efficiency traits were investigated in the study reported in Chapter 7. Using the parent-offspring regression method led to an overestimation of heritabilities, which may have been caused by common or maternal environment effects, which cause increases in the covariance between mothers and daughters (Kruuk, 2004).

8.4.3.2 Breeding worth

There was inconsistent evidence in studies looking at the relationship between feed efficiency and the New Zealand BW index. Results from the study reported in Chapter 6 suggested that high BW cows have a higher genetic potential for increased feed utilisation for the production of milk protein at a lower feed intake (Dewhurst et al. 2002), which was in contrast to findings from Woodward et al. (2011) who noted high BW animals had higher DMI and N intake. Higher milk protein at a lower intake means feed requirements of high BW animals would be lower due to their lower maintenance requirements (lower metabolic body weight) and higher efficiency of conversion of feed to milk protein.

There was a significant positive linear relationship between BW and NUE; however, BW was only a weak predictor of NUE, which is in contrast to the findings from Wheadon et al. (2012) who found a significant positive relationship between BW and NUE (r^2= 0.60) using recorded intakes. One reason for variation in this relationship is that the results used estimates of intake and efficiency that depend on the assumptions used to estimate ME requirements. However, previous correlations between estimates of DMI and NUE with measured DMI and NUE (r = 0.71 and r = 0.86 respectively; Wheadon et al. 2012) have provided some evidence that these estimates can be relatively accurate. A second reason for a weak relationship in Chapter 6 is the weightings of other traits in the BW index, some of which do not relate to components of efficiency (e.g. longevity, fertility).

The most marked effect of BW on milk production measurements was the large increase in milk protein % with increasing BW. Breeding worth is more likely to be related to processes underlying NUE than measures not directly concerning protein (e.g. fat yield) because protein yield is a dominant trait in the BW calculation, with a high economic value (NZ$8.63) compared to fat yield (NZ$1.79) and milk volume (NZ$-0.09) **(**New Zealand Animal Evaluation Limited**,** 2013) In addition, there was no difference in FCE (milk solids kg/DMI kg) between high and low BW groups in the study reported in Chapter 6 (Table 6.3). Breeding worth is more related to NUE

than FCE because whilst there are a number of factors that are common in pathways of both NUE and FCE, there are other distinctive pathways for energy or amino acid metabolism, e.g. maintenance processes that are related to the higher economic weighting on protein yield in BW.

8.4.4 Lessons learnt and limitations encountered

The research chapters in this thesis make a significant contribution to the field of study in improving the NUE of livestock and reducing environmental emissions by highlighting the use of N isotope fractionation as a proxy for predicting NUE in lactating cows and FCE in beef heifers. However, with any research it is important to consider the possible limitations and unpredicted effects that may have occurred in the research studies. The potential major limitation of using N isotope fractionation as a predictor of NUE predominantly appears to be when it is measured on animals consuming a diet high in nitrogen (Chapter 5 and 6). This increases the supply of RDP and CP, leading to an excess of N not required by the animal, which causes an increase in the amount of N converted to urea, which increases the rate of fractionation. As there is still much of N isotope fractionation that we do not know, and it is likely that protein turn over and N isotope fractionation are poorly understood, it is difficult to specifically say where N fractionation is occurring among the pathways of nitrogen utilisation, metabolism, absorption and excretion, and testing this will be a big challenge. It is particularly difficult to explain when there is an excess of N in the diet because there is an increase in N excretion, but also potentially increased N recycling. Other potential limitations are listed below.

- Variation in the pasture diet of animals in the thesis made it difficult to determine whether the between-animal variation in NUE was a result of genetic differences in utilisation of nutrients, or as a result of the differences in diet composition which led to differences in nitrogen intake.
- Practical applicability and costs of N isotope fractionation which may be expensive for use in herds with large numbers of animals (approximately $10 per

sample) and requires complex and expensive equipment to analyse isotope signatures.

- Heritability of the marker is inconclusive, therefore do not know whether it will be useful in genetic selection.
- The lack of pasture enrichment data for period 1 and very low milk enrichment in period 2 in chapter 5 causes caution in interpreting results from this chapter. In addition the inclusion of many treatment groups made it difficult to interpret underlying reasons for these results.
- Whilst identifying gaps in the literature and presenting new information regarding the relationship between NUE and N isotope fractionation, answering the initial research questions has presented more questions to be asked as a result, for example, the N isotope fractionation of essential vs. non-essential amino acids, and the microbial synthesis of essential amino acids in the rumen.

8.5 Conclusions

The main conclusions from the work reported in this thesis are;

- NUE is a more reliable and stable measurement of feed efficiency than using other energy based measures of feed efficiency (ECE) in dairy cows. NUE was highly correlated to ECE, but is less affected by the mobilisation of body reserves in early lactation so is reliable to use when evaluating the relationship between NUE and N isotope fractionation.

- The absolute maintenance requirements and the dilution of maintenance for production can help to explain the feed efficiency and N isotopic differences between dairy and beef cattle.

- There were significant negative relationships between plasma $\Delta^{15}N$ and feed efficiency (FCE or NUE) in growing beef heifers and lactating cows in two of the studies in this thesis, which with further development, may be a useful technique for use in breeding programmes, for evaluating feed efficiency without measuring feed intake (and diet composition) and comparing nutrient use efficiency of different diets. Further research is needed to understand this relationship in detecting differences for diets rich in N or RDP, which is currently a limitation of this tool.

- The high level of nitrogen in pasture was likely the main cause of weak relationships between N isotopic fractionation and feed efficiency in other studies as this increased the supply of RDP and CP. $\Delta^{15}N$ may be an indicator of the genetic variation in animal efficiency of amino acids in body tissues, but it was not related to NUE because it was diluted by the effects of excess RDP in these studies.

- Whilst a significant contribution was made to the field of study in NUE and understanding N isotope fractionation, there were some limitations, predominantly the excess of N in the diet as described above, and also in terms of

practical costs, specific sites of fractionation and variation in pasture composition.

- Preliminary analysis suggests that differences in N partitioning are a result of genetic variations in feed utilisation and nutrient metabolism. However, further investigation is required with more complex models to evaluate sire differences and relationships between parents and progeny. Excess RDP was a major contributor to the poor relationships between NUE and isotope fractionation, therefore these studies would need to be assessed with a diet with low or zero excess RDP. This approach may be difficult in situations such a New Zealand where there are high protein pastures.

- High BW was associated with more N efficient animals at a lower intake. Selection for cows based on BW may indirectly increase feed efficiency; in particular NUE, because protein yield is an important trait in the BW index and has a high economic weighting, however this process may still be slow because of genetic correlations with other traits in the index.

8.6 Future recommendations

Based on findings in this thesis, future recommendations for research are:

- Evaluating N isotopic fractionation as a biomarker for N-use efficiency in dairy cows requires further clarification. In the studies reported in this thesis, there was substantial variation in the relationship between NUE and N isotope fractionation in studies involving animals on different SR and breed groups, and studies with high CP content in the pasture. Therefore, further studies are required involving dairy cows that are (i) on a similar diet (with a focus on cows that are free-grazing) (ii), receiving diets with lower levels of CP (to reduce excess N) and (iii), not in treatment groups as this can affect efficiency and complicate relationships.

- Evidence in this thesis suggests that N fractionation is best suited to animals in similar dietary groups, therefore it can be measured within groups and then dietary groups can be compared. There is difficulty in assuming identical diets in free grazing dairy cows because of appetite behaviour and selectivity. Further work is also required to understand the temporal and spatial variations in particularly pasture which affect isotope signatures, and therefore $\Delta^{15}N$. Exploring the causes of this variation may help to reduce the effects on N isotope measurements.

- Investigate the relationship between N isotopic fractionation and feed efficiency in other species. There were differences between dairy cows and beef cattle in this thesis, suggesting that the additional pathway of milk protein synthesis alters the relationship between N isotopic fractionation and feed efficiency. It would be interesting to investigate the relationship further in animals with no rumen (e.g. pigs). Monogastrics have a simpler digestive system compared to ruminants, and the difficulties caused by excess RDP affecting the relationship between $\Delta^{15}N$ and NUE are not present.

- There was preliminary evidence to suggest that efficiency traits and N fractionation have a genetic component; however, results also suggested the need for a larger genetic study using an animal model with large numbers of animals, rather than simple sire-daughter and mother-daughter models. Despite the fact that environmental conditions were constant in these studies, estimates of heritability were unrealistically high for some traits in the study reported in Chapter 7. More complex analyses are required to account for other bias factors e.g. common or maternal environment effects, maternal and adaptive maternal effects, permanent environmental effects and genotype-environment interactions – which can all lead to mis-leading heritability estimates.

- There was inconsistent evidence in this thesis for the relationship between feed efficiency and the New Zealand BW index. In general, results showed an increase in desired production traits (particularly milk protein % and NUE) with higher BW because they have a higher genetic potential for increased feed utilisation. Breeding Worth has potential to indirectly select for increased feed efficiency within an existing selection system. However, in some studies there were weak relationships a result of weightings of other traits in the BW calculation. Therefore further analyses are required, over longer time periods to evaluate relationships with BW and feed efficiency. In addition, relationships with other indexes should be investigated e.g. Irish Economic Breeding Index (EBI).

- Investigation of gene expression in liver tissues in animals that are divergent for plasma $\delta^{15}N$ (and feed efficiency) for key genes that are up or down-regulated in important amino acid pathways. Dietary protein is the predominant driver in the rate of fractionation and most fractionation is likely to occur in liver tissues during transamination and deamination compared to rapid turnover tissues such as the small intestine. Fractionation will depend on specific types of amino acids, therefore the main sites of metabolism for each group of amino acids should be investigated in the main sites of metabolism – the liver, small intestine, rumen and mammary gland. Studies have previously found differentially expressed

genes and DNA variants between animals divergent for RFI; therefore we would expect to see different gene regulation in animals divergent for NUE. This recommendation would also test the robustness of using N isotopic fractionation for its ability to identify animals divergent for NUE.

- Study the residual effects of efficiency and N isotope fractionation across lactations and the changes in these measures repeated over longer periods of time. Lactation and pregnancy adds additional stress on the body for dairy cows, therefore during this time adaptations are made for metabolism. It would be useful to measure N isotopic fractionation sequentially over a longer period of time to understand the changes in N partitioning in transition periods. It would also be useful to further investigate the robustness of using plasma versus milk samples to measure N isotopic fractionation and their fluctuations over time.

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The author responsibilities were as follows: N. M. W. was predominantly involved in the design of each study, data analysis and preparation of the manuscript. Subsequent authors, in particular G.R.E and R.J.D were involved in the design of the studies, data analysis and contribution to manuscript writing. N. M. W. prepared the first draft of the manuscripts. All authors read and approved the final manuscripts.

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