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**The efficacy of animal-based biomarkers for nitrogen partitioning in
beef cattle**

A Dissertation
submitted in partial fulfilment
of the requirements for the Degree of
Bachelor of Agricultural Science (Honours)

at
Lincoln University
by
Jacob Ante Urlich

Lincoln University
2025

Abstract of a Dissertation submitted in partial fulfilment of the
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by

Jacob Ante Urlich

Ruminants have been associated with a negative environmental footprint, including greenhouse gas emissions and nitrogen (N) leaching. Identifying animals more efficient in N metabolism presents as a possible mitigation strategy. However, individual N efficiency requires quantification of individual animal intake, which is impractical under grazing systems. Therefore, biomarkers should be used to estimate N intake. The objective of this study was to investigate the efficacy of multiple animal-based biomarkers for N partitioning in beef Hereford heifers. 32 heifers, fed lucerne (*Medicago sativa*) silage, were measured for liveweight gained from the start of the 14 days. On four nonsequential days during the second measurement week, spot samples of blood plasma, faeces, and urine were collected in the morning (0900 h) and afternoon (1400 h). Samples collected from this were bulked across the measurement period and further analysed using nitrogen isotopic fractionation. The difference between the $^{15}/^{14}\text{N}$ (‰) content of plasma and feed was expressed as $\Delta^{15}\text{N}$. Urine N excretion (g/d) was estimated using two different equations from Chizzotti and Pacheco, giving two divergent estimations of N partitioning and use efficiency. Plasma urea N exhibited a positive relationship with urine N excretion (g/l; $r=0.52$; $P=0.003$). $\Delta^{15}\text{N}$ observed a positive correlation with both retained N efficiencies (RNE), $\text{RNE}_{\text{Chizzotti}}$ ($r=0.39$; $P=0.026$) and $\text{RNE}_{\text{Pacheco}}$ ($r=0.50$; $P=0.004$). Urinary creatinine concentrations (g/l) demonstrated a negative correlation with urine volume (l/d; $r=-0.88$; $P<0.001$), and a positive correlation with urine N concentration (g/l; $r=0.88$; $P<0.001$). $\Delta^{15}\text{N}$ presented correlations contradicting predisposed beliefs, likely due to estimation errors in intake back-calculations. Plasma urea N presents as a useful biomarker for predicting urinary N excretion in ruminants; however, care must be taken to account for diurnal variation. Creatinine aligned with previous publications, further validating its use for predicting urine output in ruminants via spot sampling.

Keywords: Retained nitrogen efficiency, nitrogen isotopic fractionation, biomarker, beef cattle, grazing, intake estimation, plasma urea, nitrogen partitioning, nitrogen excretion, creatinine, nitrogen intake.

Acknowledgements

I would like to extend my sincerest gratitude to my supervisor, Dr Omar Al-Marashdeh, for their continued feedback and support throughout this project. I would also like to thank Dr Andrew Greer, who provided invaluable preliminary advice and organised my project with Omar.

I am grateful to the laboratory staff of Lincoln University for assisting in sample preparation and analysis. Particularly, Rosy Tung, for allowing me access to the Science South laboratory and retrieving necessary equipment.

The encouragement during my study provided by my fellow students, particularly my flatmates, was greatly appreciated.

Finally, I am thankful for the support and patience from my friends and family over my Honours year.

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Chapter 1

Introduction

1.1 Introduction

The recent intensification of New Zealand agricultural systems, mainly driven by increased nitrogen fertiliser inputs and the use of irrigation (Gray, 2024), has led to an increase in stock numbers in both the dairy and beef industries, but a smaller increase in the area of land used (StatsNZ, 2024a). Sheep numbers have declined as more farms are converted to dairy systems. As a result, the environmental impact of agricultural systems has increased. These environmental impacts are largely a result of the increased nitrogen content of the consumed pasture, induced by the increased nitrogen fertiliser applications to the pasture (Beltran et al., 2022; Decau et al., 2004; Vérité et al., 2000). Pastures that have increased external nitrogen inputs typically have higher crude protein levels, leading to enhanced nitrogen intakes and thus nitrogen excretion (Waldrip et al., 2013). The main point of this excretion is the site of urination, the 'urine patch' (Decau et al., 2004). Because of this, mitigating nitrogen urinary losses is essential for reducing the environmental impacts associated with this concentration deposition onto pasture. Urinary nitrogen excretion is predicated on the nitrogen intake of the animal (Waldrip et al., 2013). Therefore, quantifying nitrogen intake per animal is essential for modelling expected urinary nitrogen losses.

Along with this, enteric agricultural greenhouse gas emissions have increased on farm as intensity has (Pinares-Patiño et al., 2009). These losses reduce the efficiency of production systems, as they increase nutrient losses on farm, meaning farmers must increase inputs to maintain production.

With nitrogen intake being a major driver for its associated excretion, quantifying an animal's nitrogen intake is critical to predicting expected urinary losses (Castillo et al., 2000). Measuring nitrogen intake has mainly occurred in zero-grazing systems with animals fed a total mixed ration (Kolver & Muller, 1998). This allows for all aspects of the forage to be controlled and, on a smaller scale, individual intake to be quantified. However, this is not practical on both a whole herd and a grazing scale, where intake can vary within these populations and systems (Herd et al., 2004). Instead of using nitrogen intake, the use of proxies presents an alternative for realistically quantifying nitrogen animal metrics (e.g., urinary excretion, use efficiency, and digestible intake) on a practical, grazing whole-herd scale (Aizimu et al., 2021).

Various biomarkers have been discussed for ruminants under a zero-grazing, total mixed ration system. These include milk urea nitrogen, neutral detergent fibre, blood urea nitrogen, and nitrogen isotopic fractionation. However, there is a lack of quantified research investigating the efficacy of the use and applicability of data for

the biomarkers when placed into a pastoral grazing context. Therefore, these markers currently have a limited field of use within New Zealand farming systems.

In place of actual measured intake data, the use of proxies presents itself as a viable alternative. This research will aid farmers in their decision-making process when considering which biomarker is the best for their system and the metric they want to measure. Quantifying the efficacy of these biomarkers for predicting urinary nitrogen excretion will benefit farmers. It will allow them to get an accurate prediction of an animal's nitrogen excretion and, therefore, wastage potential. If successful, farmers can use this to produce animals that excrete less nitrogen and use it more efficiently. This will then either increase production in the current system or allow farmers to decrease nitrogen fertiliser usage while maintaining current production values.

1.2 Objectives

The objective of this study was to investigate the efficacy of multiple animal-based biomarkers for nitrogen partitioning in beef Hereford heifers.

1.3 Hypotheses

We hypothesised that the ^{15}N isotope fractionation could present as a more effective biomarker for nitrogen use efficiency in the Hereford heifers compared with previously studied biomarkers.

Chapter 2

Literature Review

2.1 Introduction

The New Zealand agricultural industry has experienced significant increases in both overall stock numbers and intensity in recent years (MacLeod & Moller, 2006). This increase in animal numbers (because of the dairy industry expansion) increased on farm applications of nitrogenous fertiliser to match feed demands (Gray, 2024). Animal numbers increased proportionally to the nitrogen(N) fertiliser usage on farm, allowing farmers to increase the intensity of their production systems. The increase in animal numbers were

This increased intensification resulted in increased negative environmental impacts (Decau et al., 2004). With increased N fertiliser inputs, animals excrete more N back onto pastures, causing leaching during drainage events. This leaching typically happens in the form of nitrate, which is harmful to the surrounding waterways (Ball et al., 1979). This leaching has both environmental impacts but also monetary ones for the farmer, as leached N is not being converted into a product to be sold. Another environmental effect of intensification is the increased greenhouse gas emissions from livestock. With the increased livestock numbers, enteric emissions have increased on an annual total scale (Pinares-Patiño et al., 2009). These emissions are typically in the form of nitrous oxide and methane. Reducing these is desirable for farmers to reduce on farm environmental problems, such as N leaching.

With the associated problems of directly measuring a grazing animal's N intake (Aizimu et al., 2021). The use of biomarker proxies presents a possibility to circumvent the need for quantified intake data when predicting N excretion. Biomarkers vary both in their collection methods and the correlated metrics they are associated with (Fraser et al., 2025; Kohn et al., 2005; Lund et al., 2007). Therefore, choosing the correct biomarker is essential for obtaining the data with the viability for the intended purpose. This can be influenced by a multitude of factors, including class of stock, species, sex, the frequency at which they are seen, and the production system they are in. A viable marker is practical and inexpensive to collect, making the data readily available to farmers quickly and efficiently.

This review intended to describe the changes in New Zealand agriculture in terms of intensity and environmental losses. And also discuss various biomarkers for predicting nutrient excretion and use efficiency in cattle. Additionally, the associated positives and negatives of these biomarkers will be compared to one another, along with ideal uses.

2.2 Intensification of the New Zealand Agricultural Industry and Associated Problems

2.2.1 Animal Number Increase

Stats NZ(2024b) present the fluctuations in stock numbers for New Zealand from 2002 to 2024 (Figure 2.1). Figure A shows the change in stock number in dairy, beef cattle, and deer, while the change in sheep number is shown in Figure B. The significant disparity in numbers required an independent graph, adapted from (Stats NZ, 2024b). Dairy cattle numbers increased by 30% from 2002 to 2014, where numbers peaked at 6.6 million. Numbers then decreased by 13% (from 6.6 to 5.8 million) in 2024. Beef cattle, deer, and sheep numbers all decreased by 18%, 57%, and 40% respectively, from 2002 to 2024. The decrease in dairy cow numbers observed after 2021 can be explained by the implementation of the 190 kgN/ha cap. In 2021, a cap of 190 kgN/ha was placed on farms to limit N fertiliser usage to limit environmental effects (Grafton et al., 2022). This caused farmers to decrease fertiliser usage, requiring some to reduce stock numbers on farm.

These cattle numbers, presented in Figure 2.1, tie in well with the previous records of cattle production yields presented in Figures 2.2 and 2.3. These are from MacLeod & Moller (2006) and present data from 1961 to 2001. Although this period precedes the StatsNZ (2024b) data, it still provides insight into the trends occurring in the New Zealand agricultural industry. MacLeod & Moller (2006) reported that over the years from 1961 to 2001, beef production per head steadily increased till ~1994, where it dipped and then plateaued for the rest of the graph (Figure 2.2). Milk production per head saw little observed fluctuation during this period, staying at ~3000 kg/hd (Figure 2.2). In contrast to the trend in per-animal production, per-hectare production differs, as presented in Figure 3, which depicts production in per-hectare terms from 1971 to 2001 for beef (kg of liveweight) and dairy (kg of milk solids). This demonstrates a more accurate measure of the intensity of production systems. For beef production (kg of liveweight produced), the black squares rose rapidly from ~1971-1979 to ~550 kg/ha and then decreased to ~450 kg/ha and plateaued until 2001. The white squares, which are greatly lower than the black ones, were from unreliable data, which may have been swayed by changing farm classification regulations. This plateauing of beef production suggests a theoretical limit for production efficiency per land area for the period.

Contrastingly to beef, MacLeod & Moller (2006) presented that milk production per hectare steadily increased from ~5000 to ~7500 kg/ha, a 50% increase, from 1971-2001 (Figure 2.3). This steady increase in production demonstrates an increase in intensity in the dairy sector. The increase in nitrogenous fertiliser usage can partially explain this increase in intensification in the industry.

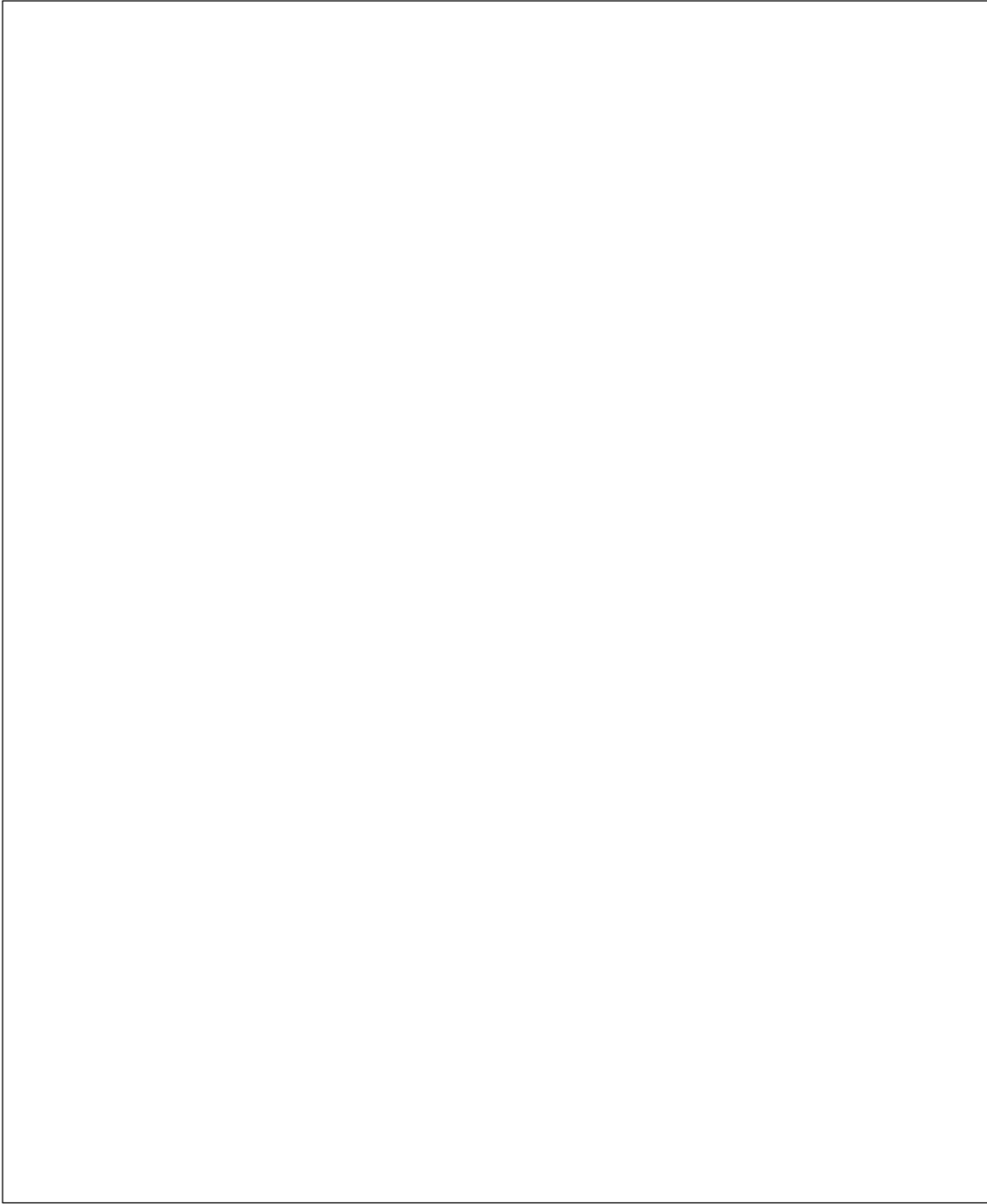


Figure 2.1: Changes in numbers of dairy and beef cattle and deer (a), and sheep (b), in New Zealand from 2002 to 2024, adapted from Stats NZ (2024b).

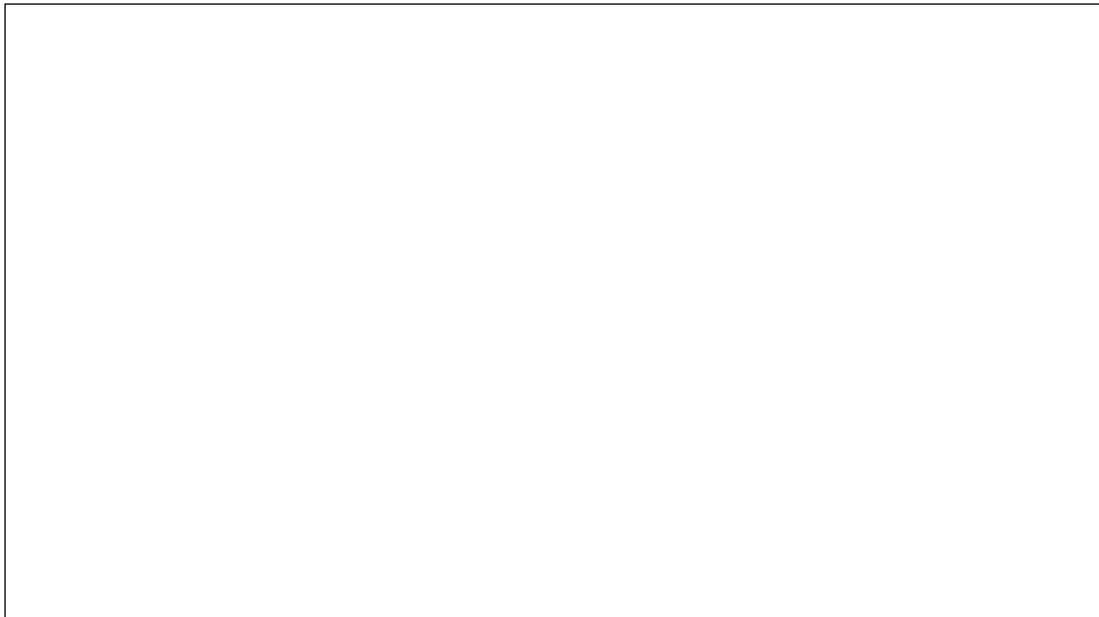


Figure 2.2: Trend in beef (■) and milk yield (o), in terms of kilograms per head, for New Zealand from 1961 to 2001 (MacLeod & Moller, 2006).

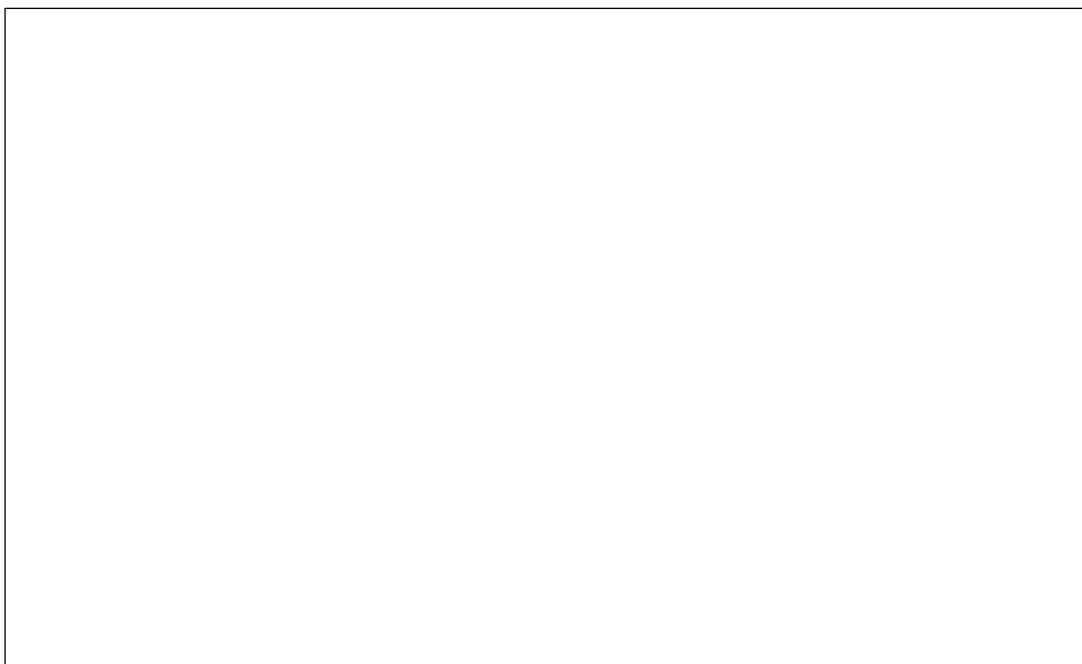


Figure 2.3: Trend in beef (■) and milk yield (o), in terms of kilograms per hectare, for New Zealand from 1961 to 2001, the open squares (□) present additional beef production statistics, but were from unreliable grasslands data (MacLeod & Moller, 2006).

2.2.2 Nitrogen Fertiliser Usage Increase

Gray (2024) presented the increased usage of nitrogenous fertiliser (Figure 2.4). The figure shows the increase in N fertiliser usage in New Zealand from 1970 to 2021. Since 1980, N fertiliser usage has increased from ~15,000 tonnes to ~475,000 tonnes in 2018/19, a ~3100% increase. After the 2018/19 season, N fertiliser usage decreased to ~440,000 tonnes, a 7% decrease. This can be attributed to the implementation of the 190 kgN/ha N cap as mentioned above, causing farmers to reduce fertiliser usage. This demonstrates that the

increased N fertiliser usage was a major catalyst for the intensification of the New Zealand agricultural industry, particularly the dairy sector. These changes are depicted in N fertiliser usage and the intensification of the agricultural industry in Gray (2024) are further supported by Rys et al. (2021), who reported similar results in fertiliser usage and intensification over this period.

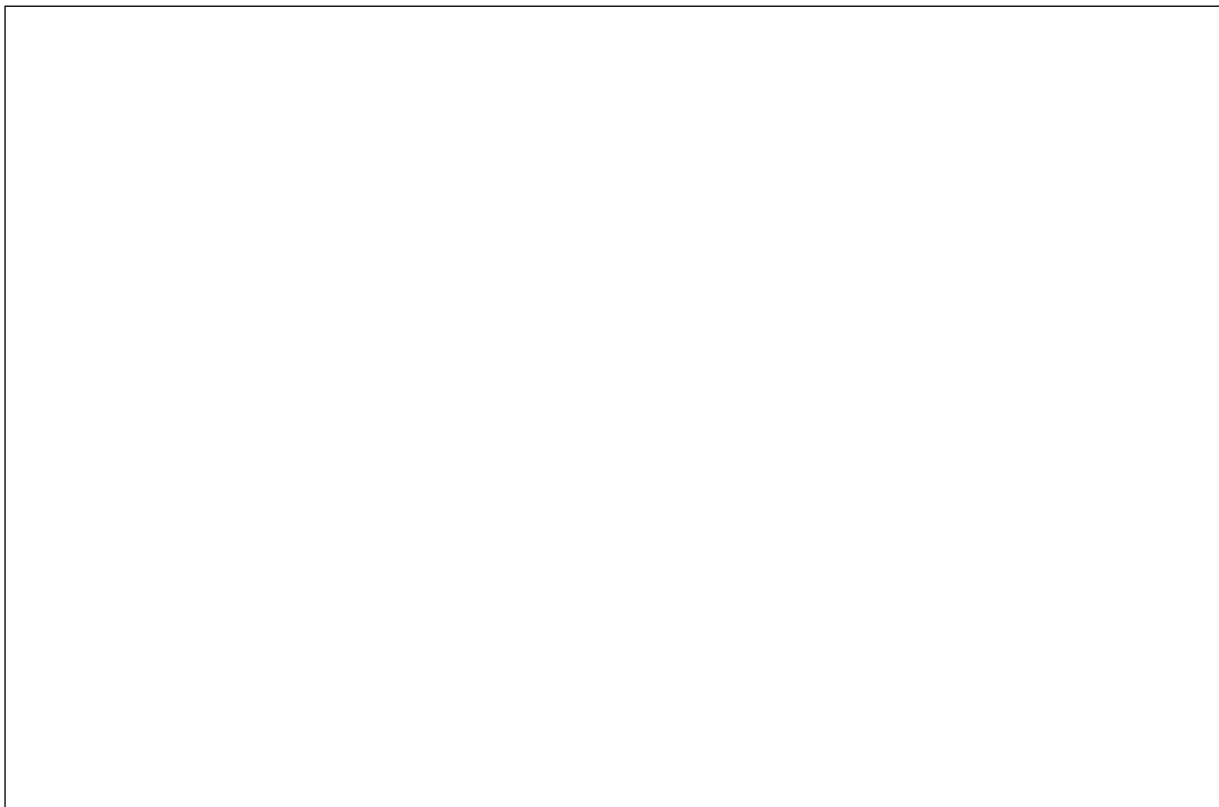


Figure 2.4: Nitrogen fertiliser consumption (bars) and cow number (line) changes in New Zealand between 1970–2021 (Gray, 2024).

2.2.3 Environmental Impact

With increased N fertiliser inputs, there are increased risks for N to be lost via leaching events (Gray, 2024; Hoogendoorn et al., 2017). Hoogendoorn et al. (2017) presented the effect of N fertiliser rates on the observed mineral N lost via leaching in a lysimeter (Figure 2.5). Increasing the N fertiliser application on the pasture increased mineral N collected in the lysimeters by ~100 mgN to ~700mgN, on average across the three years, and therefore lost via leaching in drainage events (Gray, 2024; Hoogendoorn et al., 2017). While the observed trend was similar for all years, of increased leaching with increased N fertiliser, the magnitude of the trend decreased annually. For years one, two and three, the coefficients for the lines were 1.04, 0.85, and 0.65 mg N per lysimeter per kg N fertiliser applied, respectively. The reason for this decrease in the rate of N leached was not discussed in Hoogendoorn et al. (2017).

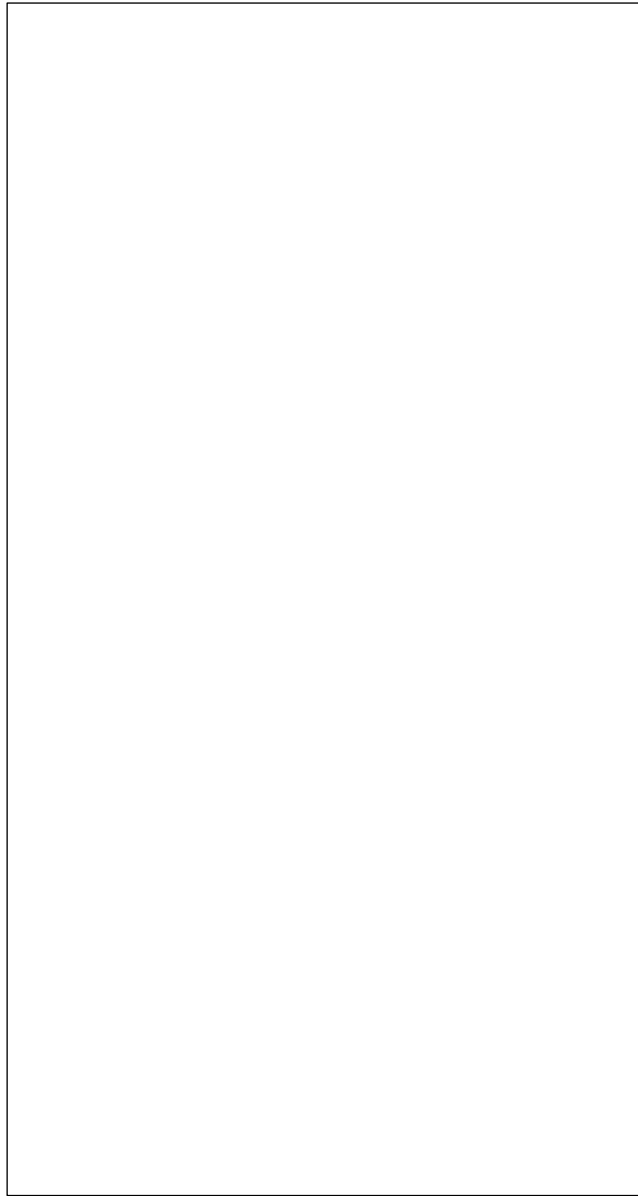


Figure 2.5: Mineral nitrogen leached relative to nitrogen fertiliser rate applied to pasture, the three lines represent the different lysimeter sites used in the trial (Hoogendoorn et al., 2017).

Gray (2024) collated nitrate leaching data from a dairy pasture over two years, from data collected by Ledgard et al. (1999; 2000) (Figure 2.6). With an increase of 0 to 200 kgN/ha/yr, there is a ~90% increase in urine N leaching and a minimal (~2 kgN/ha/yr) loss of fertiliser N recorded (Gray, 2024). Raising N applications from 200 to 400 kgN/ha/yr resulted in a ~40% increase in urine N leaching. This observed increase in N leaching aligns with Figure 2.4, which portrays a similar trend of increased N leaching losses as N fertiliser rate increases (Gray, 2024; Hoogendoorn et al., 2017). Notably, a 1900% increase (from 2 to 38 kgN/ha/yr) in fertiliser N leaching was recorded at 400 kgN/ha/yr (Gray, 2024). This highlights that the 400 kgN/ha/yr was greatly exceeding the possible N uptake of the soil. However, the more pertinent point is that even at a major extreme of N fertiliser applications (400 kgN/ha), the majority of N leaching is derived from urine patches (Gray, 2024).

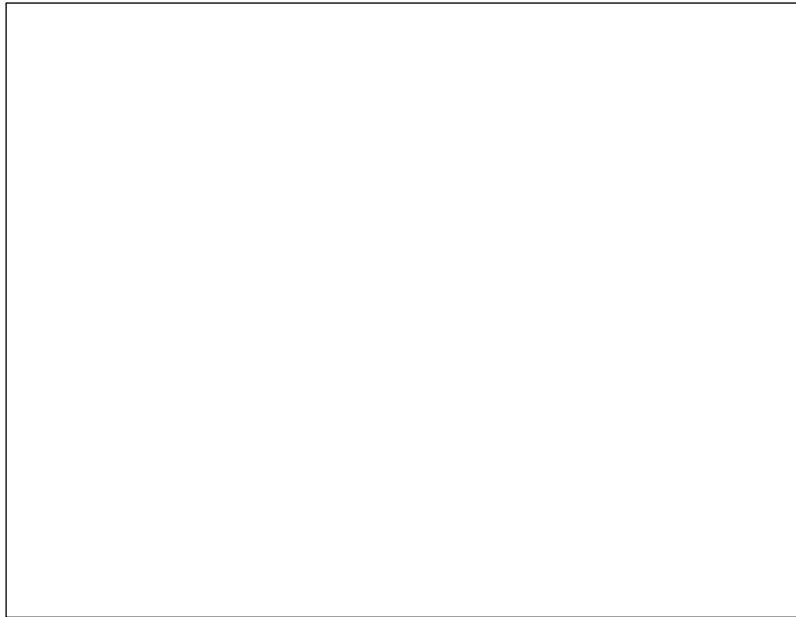


Figure 2.6: Effect of rate of nitrogen (N) fertiliser application on nitrate leaching in dairy pasture (Gray, 2024).

Decau et al. (2004) investigated the effect of N fertiliser rate (a) and urine N content (b) on cumulative nitrogen leaching (Figure 2.7). A2 and A3 urine were collected from the same animal and contained 105 and 165 kgN/ha, respectively.

For nitrogen fertiliser leaching losses (Fig. 2.7a), increasing fertiliser N content increased N leached. Increases of 30% and 75% were observed between 0-150 kgN/ha and 150-300 kgN/ha, respectively, and an overall increase of 130% from 0-300 kgN/ha (Decau et al., 2004). Cumulative winter drainage was positively correlated with N leaching losses; however, for both 0 and 150 kgN/ha, a plateau was observed at 100mm and 75mm, respectively. Looking at the gradients of the lines, it appears that there was a minimum drainage content required for N leaching to greatly increase. This minimum decreased as the N fertiliser content increased (Figure 2.7a). This trend in increasing N leaching when increasing N fertiliser application is supported by Hoogendoorn et al. (2017) and Gray (2024), who reported similar results.

In terms of urine N content affecting N leaching (Fig. 2.7b), increasing urine N content increased the N leaching of the pasture (Decau et al., 2004). Increasing urine N content from 0-105 kgN/ha resulted in a 475% increase in leached N in the pasture. For 105-165 kgN/ha, there was a 60% increase, and an overall (0-165 kgN/ha) increase of 825% in N leached. For all treatments, plateaus were observed at ~110 mm of winter drainage, resulting in 4, 23, and 37 kgN/ha leached for 0, 105, and 165 kgN/ha, respectively. A minimum drainage component to induce N leaching, like that discussed above, can be approximated at ~50 mm for all treatments. This observed trend of increased nitrogen leaching with increased urine N content is supported by Ball et al. (1979), who reported a similar increase in N leaching with increased urine N content. Notably, N leaching was comparable between the 300 kgN/ha and A3 urine and the 150 kgN/ha and A2 urine. With these

urines having lower N concentrations than the fertiliser counterparts, it highlights the increased leaching that occurs when nitrogen is deposited as urine (Decau et al., 2004; Gray, 2024).

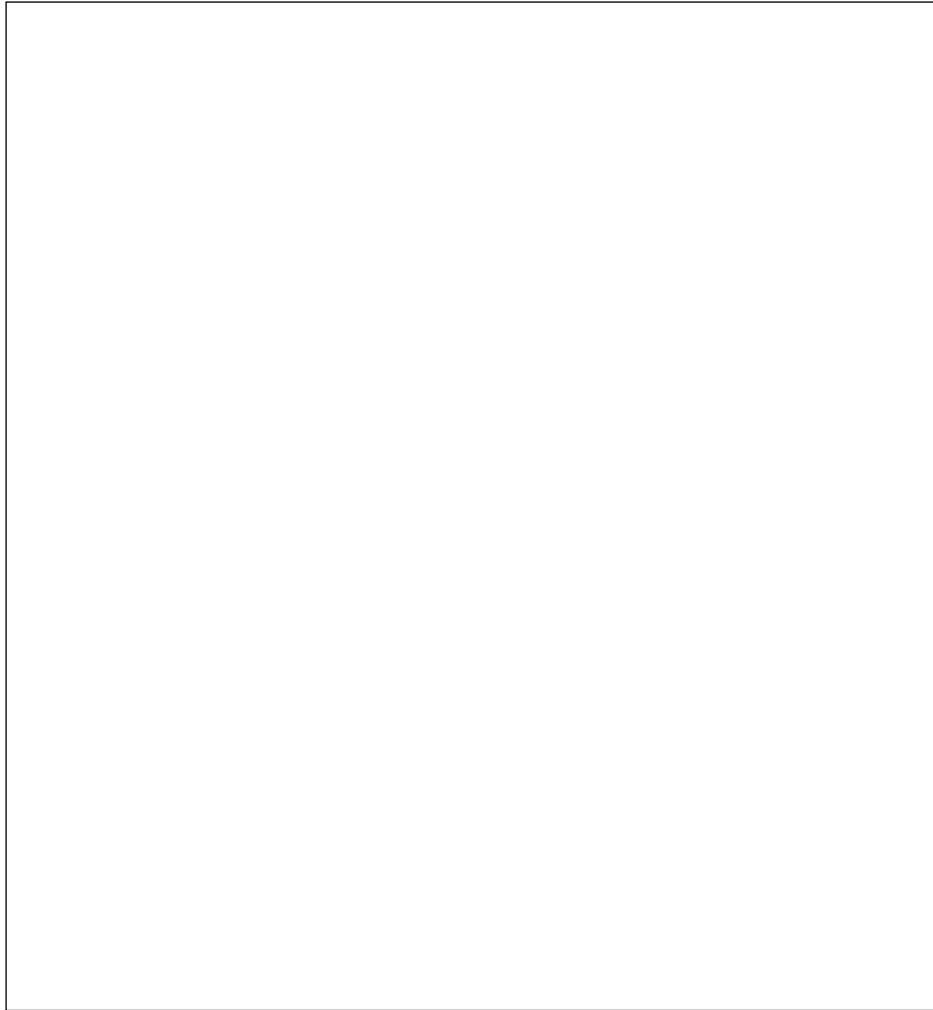
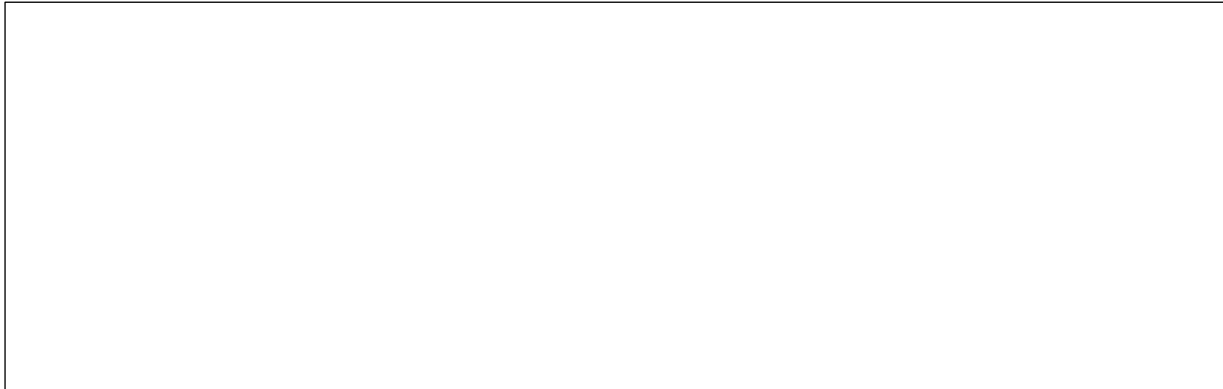


Figure 2.7: Cumulative nitrogen leaching losses for (a) mean according to fertiliser application, 0 (●), 150 (■), and 300 (▲) and (b) mean according to urine content, no added urine (●), A2 urine (■), and A3 urine (▲) (Decau et al., 2004).

The increase in cow numbers and the intensification of the agricultural industry also caused a subsequent rise in enteric greenhouse gas emissions. Pinares-Patiño et al. (2009) presented the changes in methane and nitrous oxide emissions from NZ agricultural animals between 1990 to 2006 (Table 2.1). The table presents emissions in terms of kg/year, kg/head/year, and g/kg product, which is defined as the grams of emission per output produced (for example, milk solids in dairy systems). Sheep, dairy cattle, and beef cattle stock numbers changed by -30%, 50%, and -3%, respectively. Both the beef and dairy industries have increased both methane and nitrous oxide emissions on an annual gross basis, with the sheep industry reducing, likely because of the stock number reduction. However, on a kg/head/year basis, all industries have experienced increased emissions, particularly in dairy, with a 14% and 12% increase in methane and nitrous oxide emissions, respectively. This observed increase in emissions is a direct result of the increasing intensification of the New Zealand agricultural industry discussed above. Conversely, emissions, when expressed as g/kg product, have

reduced for both the dairy and especially the sheep industry for both methane and nitrous oxide. This demonstrates that while the industries have been intensifying, they have also increased their production efficiency.

Table 2.1 Enteric methane and nitrous oxide emissions from New Zealand livestock (Pinares-Patiño et al., 2009).



2.3 Current Scene of Nitrogen and Associated Intake in Agriculture

The increasing N inputs onto pasture (Fig. 2.4) stimulate a higher N content of the pasture (Sun et al., 2008). This increased N content results in a higher crude protein content, which in turn raises the N intake of the grazing animal (Vérité et al., 2000). This increased intake has a consequence of increasing N excretion of the cattle, which increases the N leaching from farm systems (Beltran et al., 2022; Decau et al., 2004; Gray, 2024). With N leaching being largely determined by cattle excretion, limiting this excretion is essential to mitigating leaching losses on farm. Mitigation options all involve reducing the total urinary N excretion onto pasture (Beukes et al., 2017). These methods include removing the cattle from the pasture for a period and lowering the protein intake of the animal. Once removed from the pasture, animals are placed onto a pad where excrement is collected, and runoff onto pastures is stopped. This stops urine with high N concentrations from draining through the pasture, reducing N leaching. Lowering an animal's protein intake reduces total N excretion as a higher proportion of N is assimilated into tissues, i.e. an animal's N use efficiency (NUE) (Powell & Rotz, 2015). NUE is largely determined by an animal's N intake, which in turn is determined predominantly by crude protein intake; therefore, an animal's crude protein intake is a large determining factor on NUE (Katongole & Yan, 2020). Therefore, quantifying N intake of grazing animals is extremely important for determining N excretion potential and, therefore, leaching potential through the pasture. However, quantifying these intakes for grazing animals on a whole-herd scale is impossible. This impossibility of measuring wide-scale N intake arises from the variability of the overall feed intake of individuals within a cattle herd (Rumphorst et al., 2021). This limits the feasibility of obtaining N intake data for experiments conducted outside a totally controlled environment on a larger scale. Therefore, in larger experiments and on farm, alternative, indirect methods of quantifying N excretion, instead of N intake, are required. A possible

alternative for this is the use of proxy markers, which are secondary markers that correlate to these primary, overall metrics and are often more practical in a measurement sense.

2.4 Nitrogen Metabolism in Ruminants

2.4.1 Rumen Protein Degradation

Protein entering the rumen falls into two categories, rumen degradable protein (RDP) and rumen undegradable protein (RUP) (Valizadeh et al., 2021). Within the rumen, RDP degrades at varying rates, with some being instantaneous upon entering. Upon entering the rumen, the low pH of the rumen dissociates quaternary and tertiary RDP structures into simpler forms, exposing amino acids (Spek et al., 2013). Microbes break off exposed amino acids on the protein chain, extracting the nitrogen atom, and hydrogen ions, in solution, bind to form ammonia. Ammonia is toxic, and some is passed through the rumen wall into the bloodstream, where it will undergo deamination in the liver to form urea, and is excreted in urine. Ammonia remaining in the rumen will pass through the digestive tract and be excreted in the faeces. As microbes process metabolizable energy from feed, they produce proteins from the available N within the feed (Rodríguez et al., 2007). Following this microbial synthesis, the microbes die and are swept out of the rumen down to the small intestine. Once in the small intestine, the microbes are degraded, and the protein within them is absorbed into the bloodstream (Spek et al., 2013). This can account for up to 80% of an animal's protein intake. Some RUP is also absorbed in the small intestine; the rest enters the large intestine, forming an equilibrium with ammonia, and is eventually excreted in the faeces.

2.4.2 Ammonia Breakdown

Following deamination in the liver, urea is produced (Parker et al., 1995). This urea is then transported in the bloodstream to the kidneys to be excreted in the urine. Within the bloodstream, urea can readily diffuse between blood cells and plasma, forming an equilibrium (Lavery & Ferris, 2021). From this equilibrium, a plasma (blood synonymous) urea nitrogen (PUN) value can be collected by measuring the urea content of the blood. In addition to the bloodstream, urea also diffuses into the gland cistern of the udder, mixing with milk. This urea content can be measured to collect a milk urea nitrogen value (MUN) (Spek et al., 2013). Urea can also be recycled, back into the digestive system, by incorporating it into the saliva (Lavery & Ferris, 2021). This provides the urea another opportunity to be digested by the animal and utilised by tissues.

2.5 Markers for Estimating Nutrient Intake and Excretion

The biomarkers discussed below will all be animal-based; plant-derived markers will not be discussed.

2.5.1 Milk Urea Nitrogen

MUN is a measure of the urea content in the milk produced by the animal (Lavery & Ferris, 2021). MUN is a result of nitrogen degradation in a dairy cow, where the urea by-product enters the alveoli and the gland

cistern of the udder, mixing with the milk. MUN can be used as a marker for urinary N (UN) excretion and nitrogen use efficiency (NUE) (Jonker et al., 2002). MUN is a useful marker, as it can be obtained non-invasively via a bulk sample from the milk vat. This aids in both the applicability and the desire for farmer implementation, as it can be incorporated into processes already occurring on farm. Spek et al. (2013) collated data from multiple sources investigating the effect, for cattle, of MUN on UN excretion and presented them in one figure (Figure 2.8). A positive linear correlation was repeatedly observed between MUN and UN excretion. There is large inter-experiment variation, which can be attributed to both the experimental setup and individual animal variation. MUN is also negatively correlated with NUE (Lavery & Ferris, 2021).



Figure 2.8: Collated correlations of urinary nitrogen relative to milk urea nitrogen values across papers (Spek et al., 2013).

Individual variance in MUN can be explained through urea renal absorption and saliva recycling, which vary between individuals. However, this reliance on reabsorption results in an exponential increase in MUN values in diets containing high nitrogen, such as pasture-based diets (e.g. Crude Protein > 21%) (Lavery & Ferris, 2021). Diets exceeding a crude protein of 21% cause the linear relationship for MUN to UN to degrade into an exponential one, with positive and negative correlations for UN and NUE, respectively. Bodyweight is also positively correlated with MUN, as more urea is produced within the animal (Kauffman & St-Pierre, 2001). This results in an increased need for N excretion, which raises MUN levels. Being a component of milk, MUN can only be measured in lactating ruminants and, thus, alternative markers should be used in non-lactating animals. This constrains the scope of which MUN can be used both in an experimental and practical sense.

2.5.2 Blood/Plasma Urea Nitrogen

Blood urea nitrogen (BUN), similarly to MUN, is the urea content within the blood of an animal; this could also be measured in the blood plasma, which is called PUN. BUN is a result of the deamination of ammonia in the liver, producing urea, which then diffuses within the blood and associated cells. BUN is predicated on the rate of renal urea absorption and clearance, in diets where N does not exceed requirements (Kohn et al., 2005; Lavery & Ferris, 2021). BUN is therefore closely associated with urinary N and urea excretion, as when the kidneys reabsorb less urea, more enters the bladder in the urine to be excreted. Kume et al. (2008) presented the effect of plasma urea nitrogen values on observed urinary urea excretion in cattle (Figure 2.9). This demonstrates the positive relationship between PUN and UN excretion.

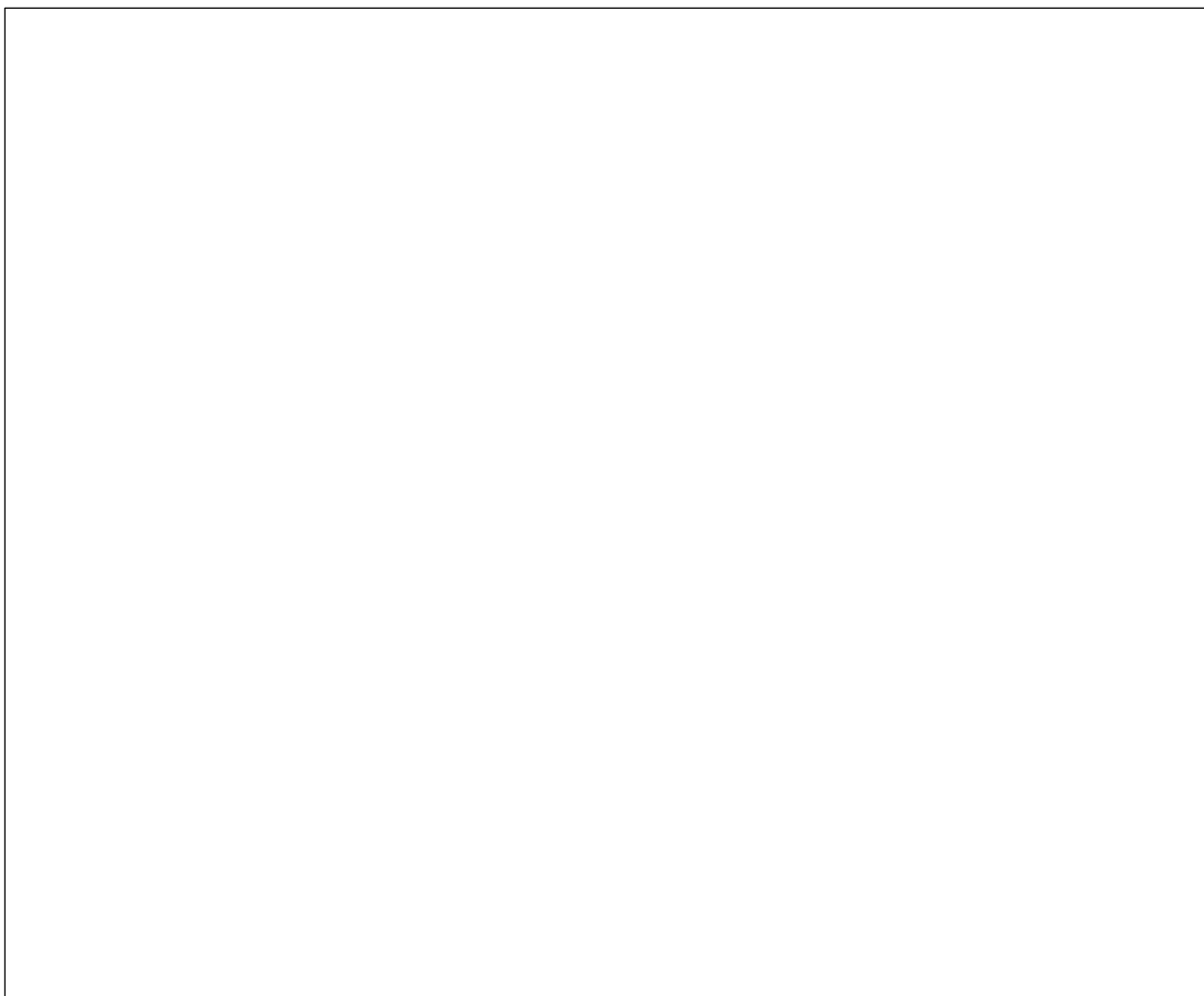


Figure 2.9: Relationship between plasma urea nitrogen and urinary urea concentration in cattle (Kume et al., 2008).

BUN fluctuates daily, relative to the time since the animal has last been fed. An animal that has been fed more recently will have more N in its digestive and circulatory system, compared with an animal that was fed longer ago (>6 hours ago), as less nitrogen will be readily available to the animal. PUN, however, is varied with dietary crude protein content (Kauffman & St-Pierre, 2001). Diets exceeding 17% crude protein were shown to have higher diurnal PUN fluctuations, as more RDP is available within the rumen, creating more ammonia and,

therefore, a larger urea spike. This increases diurnal fluctuations as PUN then lowers following filtration in the kidney and returns to pre-feed levels. Kauffman & St-Pierre (2001) investigated and presented the effect of time post-feeding for PUN at diets of 13% and 17% crude protein (Figure 2.10). No diurnal variation was observed for the 13% crude protein diet; in contrast, significant diurnal variation was observed in the 17% crude protein diet. This is further supported by Gustafsson & Palmquist (1993), who reported similar results of large diurnal PUN variations at a diet of ~17% crude protein.



Figure 2.10: Cattle plasma urea nitrogen values relative to time postprandial (post feed) at dietary crude protein levels of 13% (o) and 17% (▲) (Kauffman & St-Pierre, 2001).

BUN is also affected by the labile N pool within an animal (Lavery & Ferris, 2021; Spek et al., 2013). The labile N pool consists of N reserves in high protein usage organs (e.g. liver, kidneys) and bloodstream urea. These pools are used as a buffer, compensating for when dietary protein is insufficient to meet animal requirements. If N from the labile pool has been used, lower BUN spikes post-feeding will be observed, as dietary N will be allocated to the pool to recoup the losses, reducing the amount of urea entering the bloodstream. With BUN being a concentration measurement, blood osmolarity also affects fluctuations (Najem et al., 2024). Blood with a higher water content will reduce the overall concentration of metabolites, including BUN, and vice versa if the blood water content is low (Cheuvront et al., 2014; Najem et al., 2024). This blood osmolarity is correlated with the water intake, which, therefore, impacts BUN. BUN has been negatively correlated with nitrogen use efficiency (NUE) (Olmos Colmenero & Broderick, 2006). BUN is a possible biomarker for use; however, its large diurnal variation and invasive and labour-intensive collection methodology limit its large-scale feasibility.

2.5.3 Nitrogen Isotopic Fractionation

Nitrogen isotopic fractionation (NIF) uses the ratio of the ^{15}N isotope to the normal ^{14}N as a marker for a multitude of biological metrics and processes (Lavery & Ferris, 2021). NIF is typically expressed as the difference (Delta ($\delta^{15}\text{N}$)) between a measurable animal metric (e.g. blood plasma ^{15}N) and the feed offered to an animal (Cheng et al., 2011). This $\delta^{15}\text{N}$ can be used as a proxy marker for NUE, with a negative correlation (Cheng et al., 2013; Lavery & Ferris, 2021). Cheng et al. (2013) observed a linear negative correlation between NIF values and observed NUE in lactating dairy cows under differing levels of urea supplementation (Figure 2.11). Additionally, at the highest urea supplementation (336 g/d), the correlation broke down, which supports the claims from Wheadon et al. (2014).

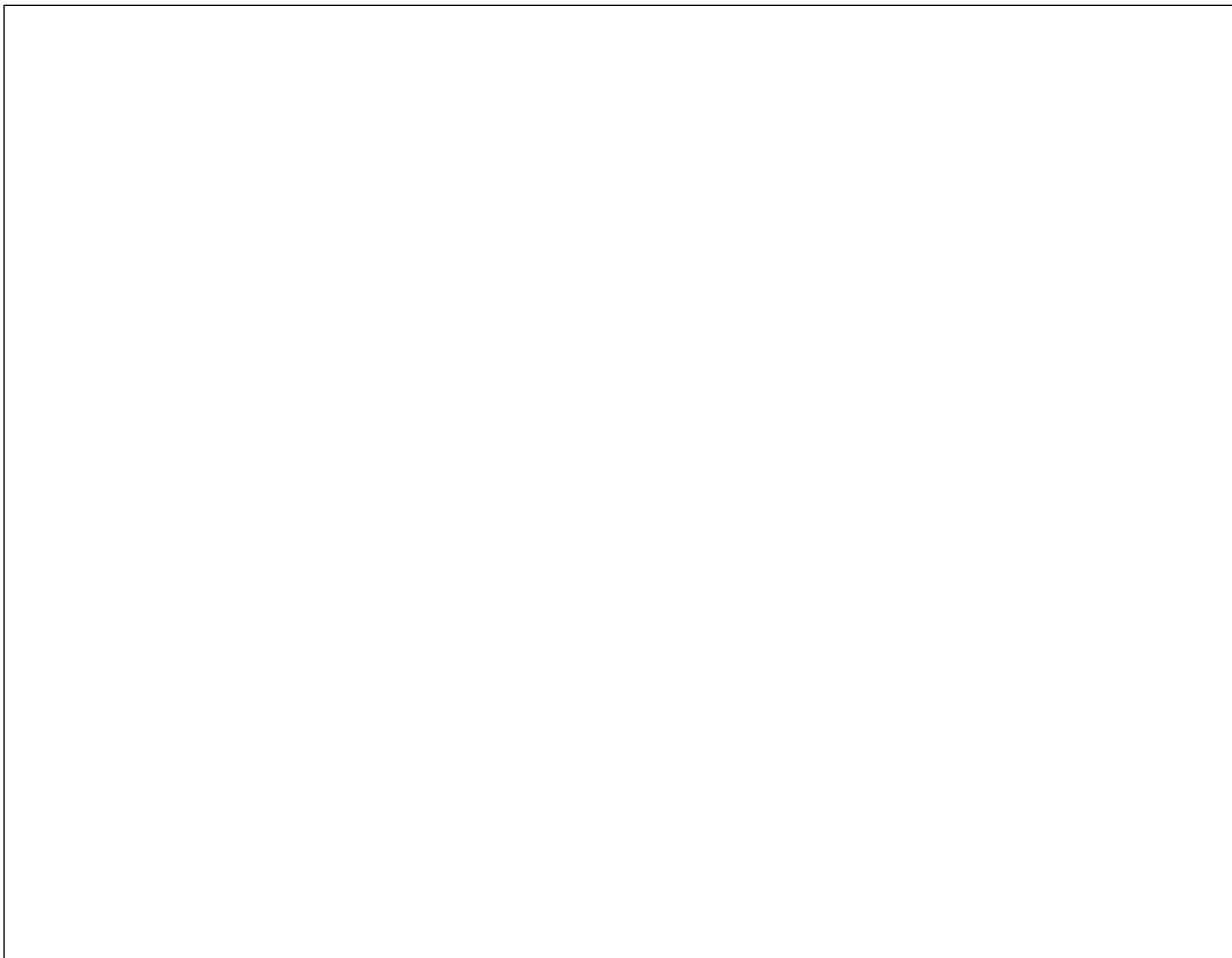


Figure 2.11: Nitrogen use efficiency (NUE) relative to nitrogen isotopic fractionation values, for milk $\delta^{15}\text{N}$ -feed $\delta^{15}\text{N}$ (open symbols) and plasma $\delta^{15}\text{N}$ -feed $\delta^{15}\text{N}$ (closed symbols), under urea supplementation of 0 g/d (Δ, \blacktriangle), 250 (\square, \blacksquare), and 336 g/d (o, \bullet) (Cheng et al., 2013).

NIF, however, has been predicted to be less reliable for estimating NUE when using $\Delta^{15}\text{N}$ of milk from animals under high rumen-degradable protein diets (Wheadon et al. 2016). These high protein levels were observed to lower the ^{15}N content of the milk, reducing the accuracy and validity of the results obtained. The $\delta^{15}\text{N}$ has also shown significant variation in accuracy and reliability in predicting values, depending on the length of data collection within a study (Cheng et al., 2011). Recently, Herremans et al. (2020) have presented that $\delta^{15}\text{N}$ may

not be a direct marker of NUE, but rather the differentiation of metabolic partitioning of N between animals and physiological stage. With the slow rate of assimilation of ^{15}N within the animal, there is no variation exhibited in values relevant to feeding time (Lavery & Ferris, 2021). Overall, $\delta^{15}\text{N}$ is a useful marker for estimating NUE for an animal and is consistent between individuals and time of collection. ^{15}N can also be collected via hair samples, compared to solely blood plasma (Fraser et al., 2025). As hair is produced on an animal, a 'timestamp' is produced, demonstrating the current isotope and hormone signatures the animal is experiencing. Hair ^{15}N is suited to being a biological marker for studies that occur over extended periods of time. This fills a niche where ^{15}N plasma or milk samples would be either economically or practically not viable to collect (Cheng et al., 2013; Fraser et al., 2025).

2.5.4 Indigestible Neutral Detergent Fibre

Neutral detergent fibre (NDF) is a component of an animal's diet, typically comprised of long structural carbohydrate chains responsible for rigid structures such as cell walls (Lund et al., 2007). This NDF can be further categorised into indigestible NDF (iNDF), which is defined as having an overall digestibility value of zero (Ellis et al., 1999). These are completely indigestible within a ruminant and will maintain their structure throughout the digestive tract and then be excreted in the faeces. iNDF can be used as a biological mark for estimating the apparent digestibility of a feed when using an initial feed of known composition. The use of iNDF as a marker for feed digestibility is well-reported on and is effective and reliably calculated (Cochran et al., 1986; Lee & Hristov, 2013; Lund et al., 2007). This need for the known diet composition is a limitation for the use of iNDF as it diminishes the applicability on farm, as feed can vary significantly between animal to animal depending on grazing pattern and behaviour, which therefore, influences feed composition and quality.

2.5.5 Creatinine

Creatinine is a product of carbohydrate metabolism in ruminants and is excreted in urine (Orr, 1918). Creatinine excretion is a function of body weight and is relatively stable across species (Chizzotti et al., 2008). Excretion is also relatively stable, irrespective of diet; it has been used to estimate total urine volume in numerous publications (Al-Marashdeh et al., 2025; Chizzotti et al., 2008). Recently, there have been investigations into the efficacy of using creatinine excretion to predict urinary nitrogen excretion. Matamura & Kondo (2025) investigated this in Wagyu cattle in Japan (Figure 2.12). The predicted urinary N excretion strongly correlated ($R^2=0.74$) with the actual observed urinary nitrogen excretion across multiple time frames. Therefore, this presents as a possible method of predicting urinary N excretion in cattle. With creatinine excretion being stable across diets, this further increases the stability of ruminant urine output predictions (Albin & Clanton, 1966). Creatinine is typically collected in spot samples, eliminating the need for total collection of urine. This makes the biomarker more desirable to use as it greatly reduces the labour and infrastructure required for collection and interpretation.



Figure 2.12: Urinary N (g/d) estimated using creatinine spot sampling relative to actual urinary N (g/d) excretion measure across time periods. 0900–1300 h, 1300–1700 h, 1700–2100 h, 2100–0100 h, 0100–0500 h, and 0500–0900 h (Matamura & Kondo, 2025).

2.6 Conclusion

Since the introduction of synthetic nitrogenous fertilizer into the New Zealand agricultural industry, cattle numbers, mainly dairy cattle, have increased while sheep numbers have declined. This has overall increased the intensity of New Zealand's agricultural production systems and heightened the associated environmental impacts of these. The increased N intake of ruminants has resulted in increased nitrogen leaching, mainly in the form of nitrates, from pastures. This leaching is directly correlated to the nitrogen content in an animal's urine, which is a product of an animal's nitrogen intake. Also, with the increased intensity, agricultural greenhouse gas emissions have increased.

Biomarkers present as a proxy for predicting nitrogen use efficiency and excretion and aiding potential mitigation strategies. These markers can be correlated with metrics of nitrogen excretion and can be used to estimate approximate excretion and, therefore, leaching potentials within a pasture. Choosing the correct biomarker depends on the species of the animal, production system, sex, and frequency of availability. Biomarkers have varying accuracies and have associated pros and cons specific to the marker. Therefore, certain biomarkers are better used in other situations depending on the circumstances.

Chapter 3

Materials and Methods

3.1 Original Dataset

Data for analysis were obtained from the dataset collected and presented by Al-Marashdeh et al. (2025). In brief, the study was conducted in South Canterbury, New Zealand, and involved 32 rising one-year-old (average age of 302 days) Hereford heifers fed lucerne (*Medicago sativa*) silage *in situ*. The study was over 14 days from the 6th to the 19th of July, 2023.

The objective of Al-Marashdeh et al. (2025) was to investigate the effect of the genetic index blood urea nitrogen breeding value (BUNBV) on the heifers' urinary N concentration. Thus, in their study, heifers were divided according to their BUNBV into a high (0.32 to 1.91) and a low (-1.95 to -0.58) BUNBV group. Each of these groups was further split into two, resulting in four groups of animals within the trial that were placed into four separate, but adjacent, pens. Animals were offered 6 kgDM of lucerne silage at ~1000 h daily with *ad libitum* water access and no other vegetation within the pens.

Daily, during the measurement period, one feed sample per pen was collected and analysed for dry matter and chemical composition. A 50g subsample was taken and oven-dried to determine dry matter content. A second subsample was taken and freeze-dried; following this, the sample was ground to <1 mm using a 1 mm sieve and then analysed by near infrared spectrophotometry (NIRS) for the chemical composition of the feed.

The study consisted of a seven-day adaptation period and a seven-day measurement period spanning from the 6th to the 19th of July 2023. All animals were weighed on day zero and 14 of the trial at ~0900-1000 h. Feed refusals were measured daily and used to calculate group feed intake as offered minus refusal. During the measurement week, blood, faecal, and urine spot samples were taken every second day (8, 10, 12, 14) in the morning (~0900 h AM) and afternoon (~1400 h PM). Faecal samples were analysed similarly to feed, see above, for nitrogen concentration. Urine samples, once collected, were acidified to prevent instantaneous ammonia volatilisation and were analysed for urinary nitrogen, urea, and creatinine values.

3.2 New Data Measurement and Analysis

3.2.1 ¹⁵N Analysis

Both feed and plasma samples were bulked per animal across the sampling times and days. For plasma, a 0.25 ml subsample per sample was taken to provide a 1.5 ml total sample per animal, across morning and afternoon, for the whole measurement period. Plasma samples were then freeze-dried and stored at room temperature for analysis. For feed, the freeze-dried samples were ground to <1 mm using a 1 mm sieve before

being bulked across sampling day on a per pen basis. Bulked feed samples were then sent for ^{15}N analysis. For ^{15}N analysis, samples were analysed using an isotope ratio mass spectrometer. The upper and lower acceptable reference values for air $\delta^{15}\text{N}$ were 47.56‰ and -4.52‰. Measured values were -4.51‰ and 47.57‰, within acceptable limits. The results from this are expressed as delta units relative to the air ($\delta^{15}\text{N}$) in parts per 1000 (‰). $\delta^{15}\text{N}$ is a measure of the ^{15}N content of a sample, relative to the ^{15}N content of the air, and these are expressed as ratios of $^{15}\text{N}/^{14}\text{N}$. The difference between the $\delta^{15}\text{N}$ plasma and $\delta^{15}\text{N}$ feed will be reported and discussed as ' $\Delta^{15}\text{N}$ '.

3.2.2 Individual Animal Dry Matter Intake Measurements

Individual animal dry matter (DM) intake was back-calculated based on the metabolisable energy (ME) of each heifer using equations in Rattray et al. (2017). Basal maintenance (Mt) requirements were calculated for each animal using equation 1 in Rattray et al. (2017) as below:

Equation 1: Basal maintenance energy requirements (Rattray et al., 2017)

$$ME_b = \text{Species} * \text{sex} * 0.28 * \text{EXP}(-0.03 * \text{age in years}) * (\text{LWT}^{0.75})/k_m$$

Where: LWT = average liveweight over measurement period (kg)

Species = 1.3 (beef cattle)

Sex = 1.0 (females)

$k_m = \text{M/D} * 0.02 + 0.5$

M/D = ME concentration of feed (MJME/kgDM)

With animals being fed in situ, grazing and walking activity were assumed to be zero.

The energy required for the measured liveweight change was calculated using the equation below.

Equation 2: Liveweight gain energy requirements (Rattray et al., 2017).

$$ME = 1.1 * \text{LWG} * \frac{NE_g}{k_g}$$

Where: LWG = liveweight gain (kg/day)

$$NE_g = (0.92 * \text{LWG}) * \left(\left(6.7 + \left(\left(\frac{920 * \text{LWG}}{4 * (\text{SRW}^{0.75})} \right) \right) - 1 \right) \right) + (20.3 - \left(\left(\frac{920 * \text{LWG}}{4 * (\text{SRW}^{0.75})} \right) \right) - 1) / (1 + \text{EXP}(-6 * \left(\left(\frac{\text{LWT}}{\text{SRW}} \right) - 0.4 \right)))$$

Where: SRW = standard reference weight (kg) = 600 kg (Adjabui et al., 2025)

$k_g(\text{dry}) = (\text{M/D} * 0.042) + 0.006$

The DM intake was then calculated by dividing estimated energy requirements for both maintenance and liveweight gain by the metabolisable energy value of lucerne silage. The N intake was calculated as DM intake × N content of lucerne silage.

3.3 Excretion Calculations

The total faecal excretion per animal (kg/day) was estimated using the following equation.

Equation 3: Total faecal excretion per day (Adjabui et al., 2025)

$$Faecal\ DM \left(\frac{kg}{d} \right) = DMintake \left(\frac{kd}{d} \right) * \left(1 * \frac{DMD\%}{100} \right)$$

Where: DMD is the dry matter digestibility of the lucerne silage.

Faecal N excretion (g/d) was then calculated by multiplying faeces volume kg DM/day by faecal N content (g/kg DM)

The total urine volume was estimated using two different equations. From this, two different values for urine N g/d were calculated: Urine_{Chizzotti} N g/d using the Chizzotti et al. (2008) (Equation 4), and Urine_{Pacheco} N g/d using the Pacheco et al. (2007) (Equation 5). Subsequently, two values of retained N g/d were also calculated. 'Retained_{Chizzotti} N g/d' using urine N g/d was calculated based on Chizzotti et al. (2008), and 'Retained_{Pacheco} N g/d' using urine N g/d calculated based on Pacheco et al. (2007).

Equation 4: First urine volume equation from Chizzotti et al. (2008).

$$Urine\ volume \left(\frac{l}{d} \right) = (0.28 - (0.000097 * LWT)) * \frac{LWT}{UCC}$$

Where: LWT = Average liveweight (kg)

UCC = Urine creatinine concentration (mmol/L)

Equation 5: Urinary nitrogen excretion equation from Pacheco et al. (2007).

$$Urinary\ Nitrogen \left(\frac{gN}{d} \right) = \left(\frac{21.9 * LWT}{UC} \right) * UN$$

Where: UC = Urinary creatinine (mg/L)

UN = Urinary nitrogen concentration (g/L)

Retained N values were calculated using the following equation.

Equation 6: From Spanghero & Kowalski (2021).

$$\text{Retained } N \left(\frac{g}{d} \right) = N_{\text{intake}} - (N_{\text{faeces}} + N_{\text{urine}})$$

From there, RNE can be calculated.

Equation 7: Retained nitrogen efficiency (RNE) equation.

$$RNE\% = \left(\frac{\text{Retained } N}{N \text{ intake}} \right) * 100$$

3.4 Statistical analysis

Data analysis was performed using both Genstat v 24 and RStudio statistical software packages. Genstat was used for the linear regression of the biomarkers and responses, and for producing figures. Linear regression analysis was conducted using both $\delta^{15}\text{N}$ Plasma – $\delta^{15}\text{N}$ Feed and PUN values as independent variables. Urinary N excretion and NUE values were used as dependent variables during analysis. RStudio generated the Pearson correlation figures. Within the dataset, an outlier within Retained_{Pacheco} N g/d was discovered for animal number 220074. This number, being 365 g N/d retained, was determined to be biologically invalid and was thus excluded from the dataset for analysis. Therefore, the subsequent RNE_{Pacheco} value was omitted from the dataset as it was unable to be calculated for this animal. Significance was stated at $P < 0.05$.

Chapter 4

Results

Chemical composition of the lucerne fed to the heifers is presented in Table 4.1. Average crude protein content was 25.5%, metabolisable energy was 9.9 MJME/kgDM, and NDF was 41.8%. Back-calculated estimates and measure variables were presented in Table 4.2. Average DM intake was 5.0 kgDM/d, N intake was 207.2 g/d, digestible N intake was 165.8 g/d, and N digestibility was 80.1%. The average urine N concentration was 8.6 g/l, urine urea was 16.0 g/l, daily urine_{Chizzotti} N excretion was 117.4 g/d, and urine_{Pacheco} N was 88.0 g/d. Average $\Delta^{15}\text{N}$ was 4.57, urine creatinine concentration was 0.45 g/l, RNE_{Chizzotti} was 8.7%, and RNE_{Pacheco} was 26.5%.

Table 4.1: Chemical composition of the lucerne silage fed to heifers.

Nutrient	Mean \pm SD
Crude Protein %	25.5 \pm 1.29
Neutral Detergent Fibre %	41.8 \pm 3.17
Metabolisable Energy MJME/kgDM	9.9 \pm 0.41
N Content g/kgDM	40.7 \pm 2.07
Dry Matter Digestibility %	67.0 \pm 2.59

Table 4.2: Statistical summary of the variates used in statistical analysis.

Measured/Estimated Variable	Mean	SD	Min	Max
Intake kg DM/d ¹	5.0	2.71	2.6	13.9
N intake g/day ²	207.2	111.06	104.9	568.9
Faecal excretion kg DM/d	1.7	0.89	0.8	4.6
Faecal N content g/kg	24.8	2.03	20.3	28.5
Faecal N excretion g/d	41.3	21.8	20.1	101.5
Plasma urea N mg/dl ³	19.9	1.92	17.1	25.8
Urine volume l/d	14.5	4.3	7.9	27.1
Urine N concentration g/l	8.6	1.83	5.1	12.2
Urine _{Chizzotti} N g/d ⁴	117.4	15.91	92.7	145.5
Urine _{Pacheco} N g/d ⁵	88.0	11.00	69.2	109.7
Urine urea concentration g/l	16.0	3.54	9.7	22.6
Urine urea excretion g/d	219.7	30.21	178.0	277.6
Urine creatinine concentration g/l	0.45	0.121	0.23	0.79
Urine N to Creatinine ratio g/g	35.9	4.07	25.9	42.9
$\delta^{15}\text{N}$ Plasma – $\delta^{15}\text{N}$ Feed ⁶	4.57	0.239	4.09	5.12
Retained _{Chizzotti} N g/d ⁷	48.4	92.14	-59.1	353.7
Retained _{Pacheco} N g/d ⁸	68.5	70.26	-8.4	208.3
Digestible N g/d	165.8	89.64	84.1	467.4
N digestibility %	80.1	1.63	77.1	83.7
Liveweight kg ⁹	210	18.6	175	242
Liveweight gain kg ¹⁰	8.2	6.71	-2.0	22.0
RNE _{Chizzotti} % ¹¹	8.7	31.3	-56.3	62.2
RNE _{Pacheco} % ¹¹	26.5	19.80	-6.6	58.4

¹DM= Dry matter; ²N= Nitrogen; ³Plasma urea nitrogen = PUN; ⁴Urinary N excretion (g/d) calculated using urine volume equation from Chizzotti et al. (2008); ⁵Urinary N excretion (g/d) calculated using the equation from Pacheco et al. (2007); ⁶ $\delta^{15}\text{N}$ Plasma – $\delta^{15}\text{N}$ Feed will be discussed as ' $\Delta^{15}\text{N}$ ' within the results section; ⁷N retained in liveweight was calculated as total N intake minus N excreted in faeces and urine with urine N calculated based on Chizzotti et al. (2008) equation; ⁸N retained in liveweight was calculated as total N intake minus N excreted in faeces and urine with urine N calculated based on Pacheco et al. (2007) equation; ⁹Liveweight average across the 14 day trial period; ¹⁰Liveweight gain (LWG) across the 14 day trial period; ¹¹Retained N Efficiency (RNE) was calculated by dividing the respective retained N by N intake, then expressing as a percentage.

4.1 Biomarker Correlations

For $\Delta^{15}\text{N}$, no significant correlations ($p>0.05$) were found for any of the urinary nitrogen excretion metrics (Figure 4.1). $\Delta^{15}\text{N}$ was shown to moderately correlate with retained_{Chizzotti} N (g/d) ($r=0.36$; $P=0.046$), retained_{Pacheco} N (g/d) ($r=0.42$; $P=0.018$), digestible N (g/d) ($r=0.42$; $P=0.019$), RNE_{Chizzotti} (%) ($r=0.39$; $P=0.028$), and RNE_{Pacheco} (%) ($r=0.50$; $P=0.004$; Figure 4.1). $\Delta^{15}\text{N}$ also moderately correlated with faecal N (g/d) ($r=0.4$; $P=0.024$), and with intake (kgDM/d), N intake (g/d), and total faeces DM (kg/d), all with identical correlations ($r=0.98$; $P<0.001$; Figure 4.1).

PUN (mg/dl) concentration was shown to moderately correlate with urine N concentration (g/l) ($r=0.53$; $P=0.003$), and urine urea concentration (g/l) ($r=0.58$; $P<0.001$; Figure 4.1). A moderate correlation with PUN (mg/dl) and urine creatinine concentration (g/l) ($r=0.5$; $P=0.006$), and faecal N (g/d) ($r=0.43$; $P=0.005$; Figure

4.1), were also observed. Additionally, moderate correlations between PUN (mg/dl) and N digestibility (%) ($r=-0.49$; $P=0.005$), and PUN and urine volume (l/d) ($r=-0.44$; $P=0.014$; Figure 4.1) were found.

N intake (g/d) was shown to strongly correlate with $RNE_{Chizzotti}$ (%) ($r=0.9$; $P<0.001$), $RNE_{Pacheco}$ (%) ($r=0.92$; $P<0.001$), $retained_{Chizzotti}$ N (g/d) ($r=0.9$; $P<0.001$), $retained_{Pacheco}$ N (g/d) ($r=0.92$; $P<0.001$), faecal N concentration (g/kg) ($r=0.99$; $P<0.001$), and LWG ($r=0.49$; $P=0.006$; Figure 4.1). N intake (g/d) was the sole determining factor for digestible N (g/d) ($r=1$; $P<0.001$) and total faeces DM (kg/d) ($r=1$; $P<0.001$; Figure 4.1). This is because, along with N intake, variation within estimated data was solely resulting from intake (kgDM/D) back-calculations; thus, all data observed identical variation.

Urine creatinine concentration (g/l) was shown to strongly correlate with urine volume (kg/d) ($r=-0.88$; $P<0.001$), urine N concentration (g/l) ($r=0.88$; $P<0.001$), and urine urea concentration ($r=0.86$; $P<0.001$; Figure 4.1). Moderate correlations with urine creatinine concentration (g/l) were also found for $urine_{Chizzotti}$ N (g/d) ($r=-0.51$; $P=0.003$), $urine_{Pacheco}$ N (g/d) ($r=-0.44$; $P=0.014$), and urine urea excretion (g/d) ($r=-0.44$; $P=0.013$; Figure 4.1).

Urine N to creatinine ratio (g/g; UNCR) was shown to moderately correlate with urine volume (l/d) ($r=0.38$; $P=0.033$), $urine_{Chizzotti}$ N (g/d) ($r=0.67$; $P<0.001$), and $urine_{Pacheco}$ N (g/d) ($r=0.63$; $P<0.001$).

$RNE_{Chizzotti}$ (%) was strongly correlated with intake (kgDM/d) ($r=0.9$; $P<0.001$), N intake (g/d) ($r=0.9$; $P<0.001$), total faeces DM (kg/d) ($r=0.9$; $P<0.001$), faecal N excretion (g/d) ($r=0.89$; $P<0.001$), $Retained_{Chizzotti}$ N (g/d) ($r=0.95$; $P<0.001$), and digestible N intake (g/d) ($r=0.9$; $P<0.001$; Figure 4.1). $RNE_{Chizzotti}$ (%) also moderately correlated with $urine_{Chizzotti}$ N excretion (g/d) ($r=-0.36$; $P=0.045$; Figure 4.1).

$RNE_{Pacheco}$ (%) strongly correlated with intake (kgDM/d) ($r=0.92$; $P<0.001$), N intake (g/d) ($r=0.92$; $P<0.001$), total faeces DM (kg/d) ($r=0.92$; $P<0.001$), faecal N concentration ($r=0.96$; $P<0.001$), $Retained_{Pacheco}$ N (g/d) ($r=0.95$, $P<0.001$), and digestible N intake (g/d) ($r=0.93$, $P<0.001$; Figure 4.1).

Urine urea concentration (g/l) was moderately correlated with N digestibility (%) ($r=-0.47$; $P=0.008$). Urine volume (l/d) was strongly correlated with urine N concentration ($r=-0.84$; $P<0.001$) and urine urea concentration (g/l) ($r=-0.84$; $P<0.001$; Figure 4.1), on account of them being concentration metrics. Urine volume (l/d) also moderately correlated with $urine_{Chizzotti}$ N (g/d) ($r=0.63$; $P<0.001$), $urine_{Pacheco}$ N (g/d) ($r=0.41$; $P=0.023$), and urine urea excretion (g/d) ($r=0.54$; $P=0.002$; Figure 4.1).

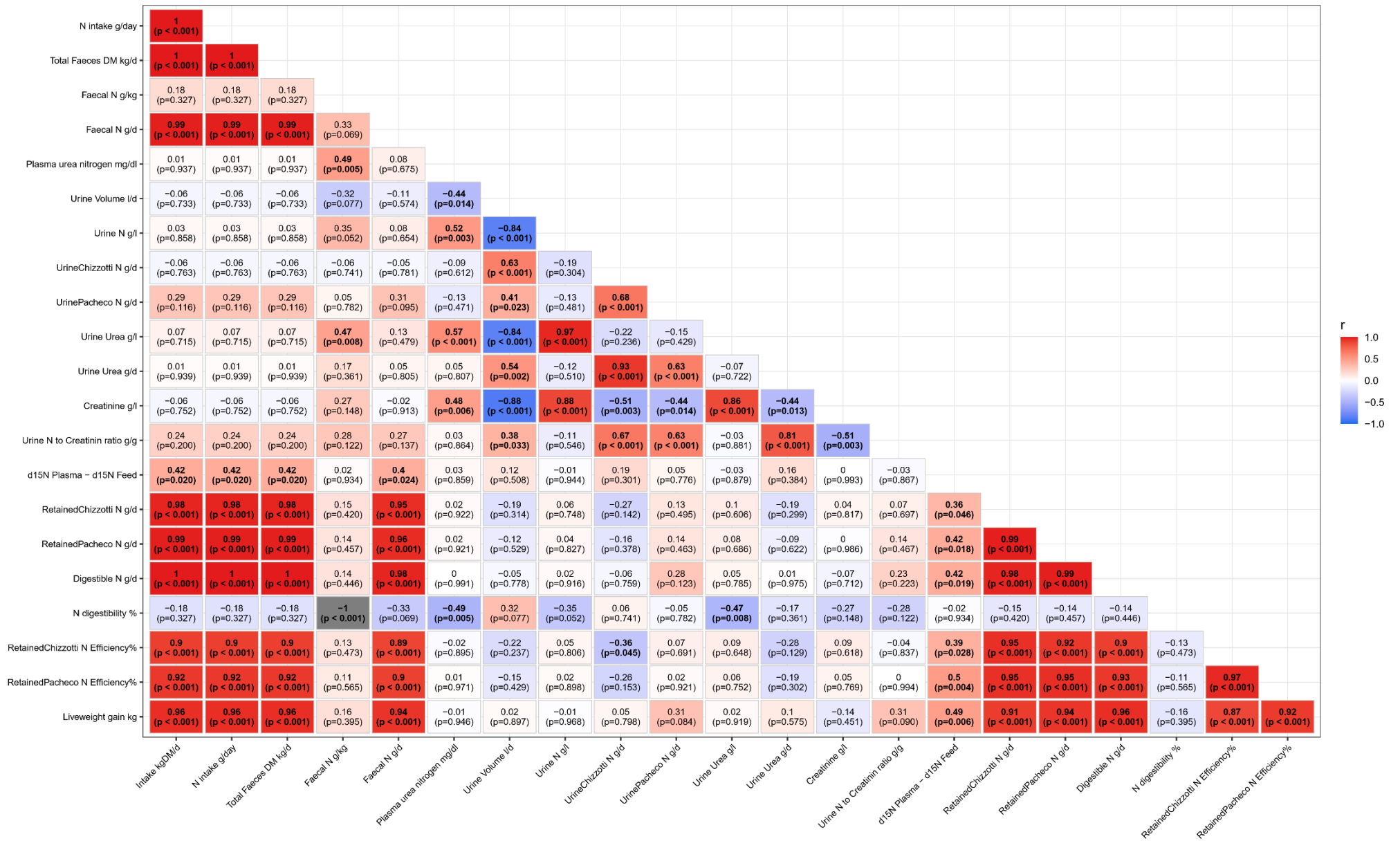


Table 4.3: Pearson correlation table for biomarkers to various animal metrics, presenting r values and p-values. The δ character for the ^{15}N analysis would not export with the JPG from RStudio and thus was replaced with d.

4.2 Linear regression

The correlation between PUN (mg/dl) and urine N concentration (g/l) was further analysed with a simple linear regression model (Figure 4.1). The regression was significant ($P=0.002$) with an R^2 of 0.26, giving a regression equation of urine N = $-1.60 + 0.509 \cdot \text{PUN}$ (mg/dL). A one unit increase in PUN is predicted to result in an increase in urine N concentration by 0.509 g/l.

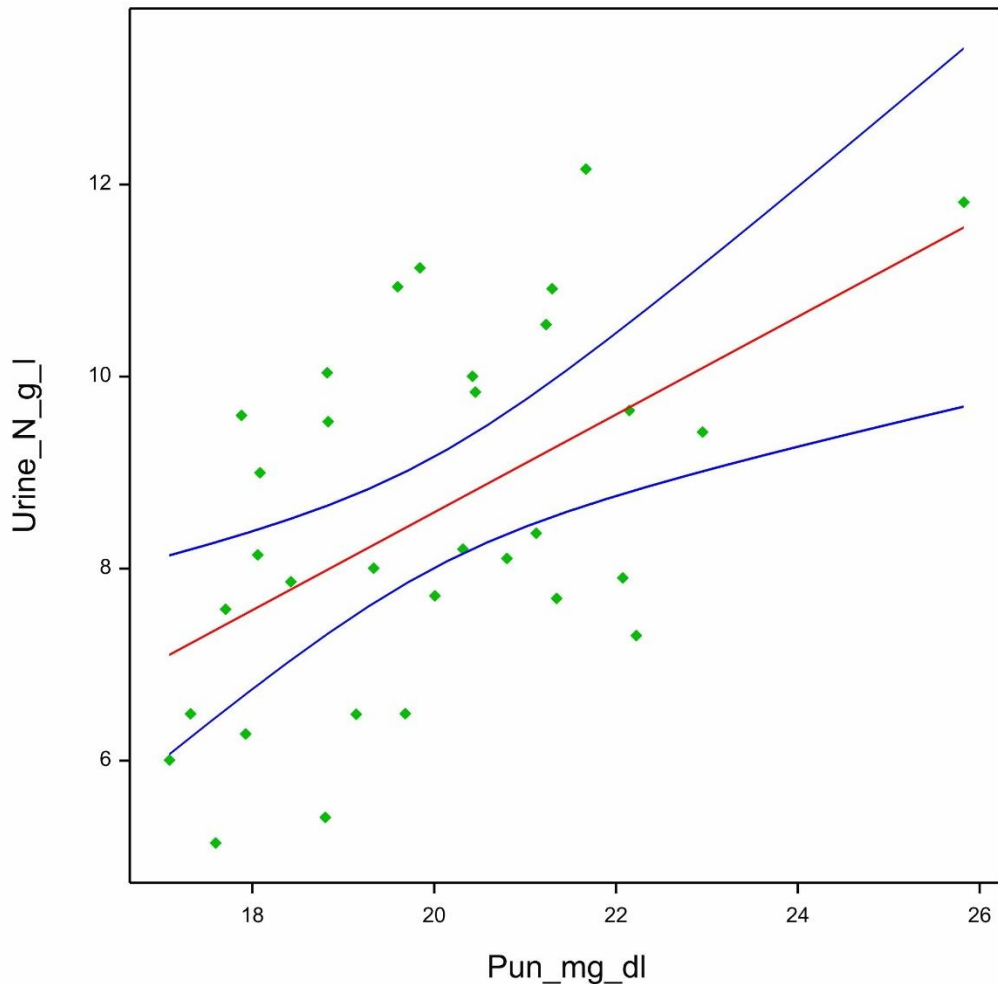


Figure 4.1: Urine nitrogen concentration (g/l) of Hereford heifers relative to plasma urea nitrogen (PUN) concentration, with 95% confidence intervals. Regression model $R^2=0.26$ ($P=0.002$).

A simple linear regression model was run on RNE (%) relative to $\Delta^{15}\text{N}$, with RNE values grouped by the urine N excretion values used (i.e., Chizzotti or Pacheco; Figure 4.2). The model was statistically significant ($P<0.001$) with an R^2 of 0.273. No statistically significant difference was found between the RNE values used ($P=0.436$). $\Delta^{15}\text{N}$ was a statistically significant ($P<0.001$) predictor of RNE. The regression equations for the model were $\text{RNE}_{\text{Chizzotti}} = -2.716 + 0.613 \cdot \Delta^{15}\text{N}$, and $\text{RNE}_{\text{Pacheco}} = -1.776 +$

0.449 * $\Delta^{15}\text{N}$ (Figure 4.2). A one unit increase in $\Delta^{15}\text{N}$ is predicted to increase $\text{RNE}_{\text{Chizzotti}}$ and $\text{RNE}_{\text{Pacheco}}$ by 61.3% and 44.9%, respectively.

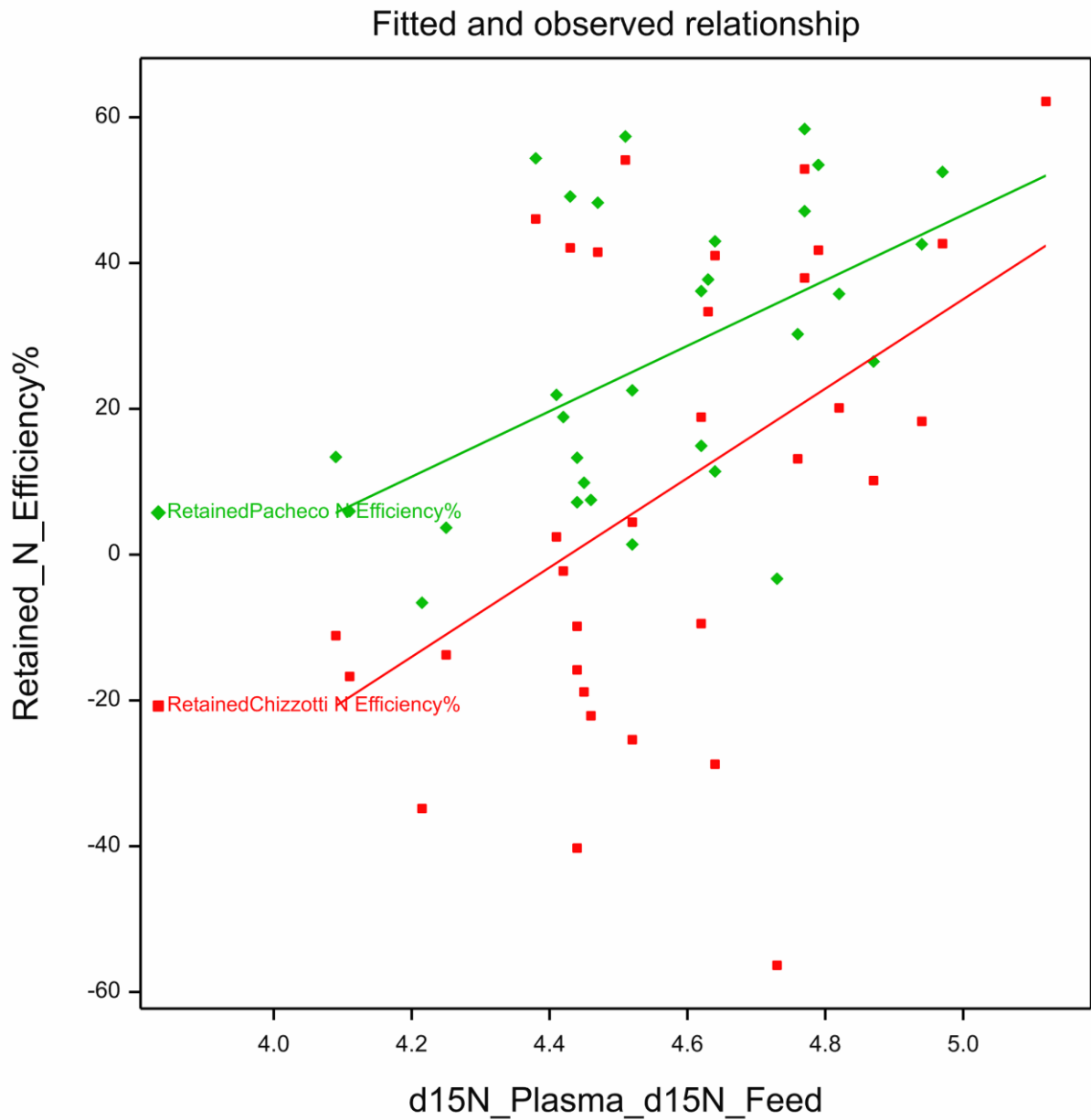


Figure 4.2: Retained N efficiency (RNE) (%) for Hereford heifers eating lucerne silage relative to $\delta^{15}\text{N}$ Plasma – $\delta^{15}\text{N}$ Feed values ($d=\delta$). RNE values are grouped by urine nitrogen excretion (g/d) values used, giving $\text{RNE}_{\text{Chizzotti}}$ (%) (■) and $\text{RNE}_{\text{Pacheco}}$ (%) (◆). Regression model $R^2=0.273$ ($P<0.001$).

A regression model of LWG (kg) relative to $\Delta^{15}\text{N}$ was run (Figure 4.3). A moderate and significant correlation was found between the terms ($R^2=0.307$; $P<0.001$), producing a regression equation of $\text{LWG} = -64.1 + 15.81 * \Delta^{15}\text{N}$. A one unit increase in $\Delta^{15}\text{N}$ predicts an increase in LWG by 15.81 kg.

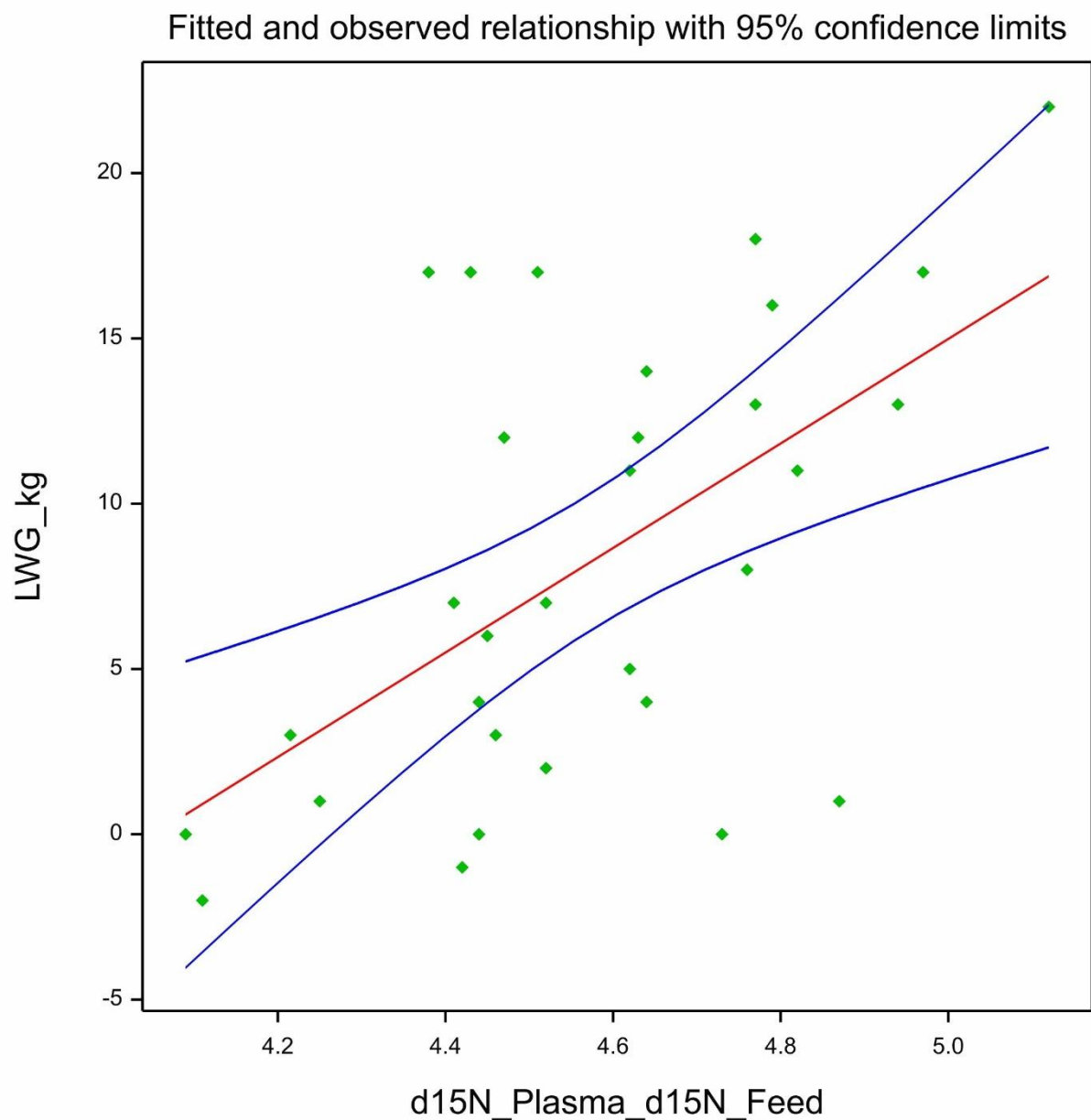


Figure 4.3: Liveweight gain (LWG; kg) of rising one-year-old Hereford heifers fed lucerne silage across 14 days relative to average $\delta^{15}\text{N}$ Plasma – $\delta^{15}\text{N}$ Feed ($\delta=d$). Regression model $R^2=0.307$ ($P<0.001$).

Following this, a regression model was run between LWG, and $\text{RNE}_{\text{Chizzotti}}$ and $\text{RNE}_{\text{Pacheco}}$ (Figure 4.4). The model was strong and significant ($R^2=0.801$; $P<0.001$). The regression equations were as follows: $\text{LWG} = 6.540 + 18.99 * \text{RNE}_{\text{Chizzotti}}$ and $\text{LWG} = -0.020 + 29.27 * \text{RNE}_{\text{Pacheco}}$. A one unit increase in RNE is predicted to increase LWG by 18.99 kg and 29.27 kg for $\text{RNE}_{\text{Chizzotti}}$ and $\text{RNE}_{\text{Pacheco}}$, respectively.

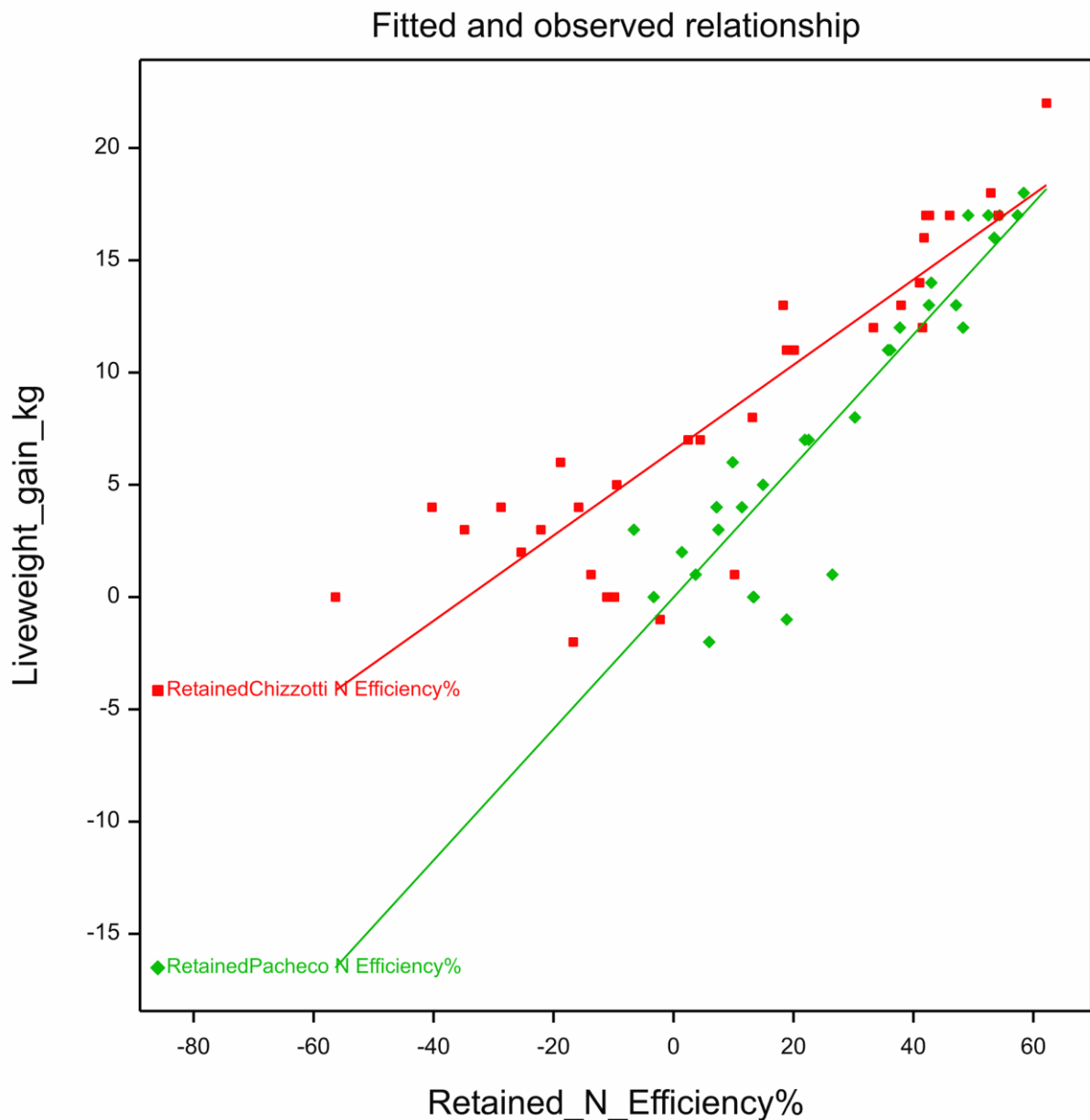


Figure 4.4: Liveweight gain (LWG; kg) of rising one-year-old Hereford heifers fed lucerne silage over 14 days relative to retained N efficiency (RNE; %). RNE values are grouped by urine nitrogen excretion (g/d) values used, giving RNE_{Chizzotti} (%) (■) and RNE_{Pacheco} (%) (◆). Regression model $R^2=0.801$ ($P<0.001$).

Furthermore, RNE_{Chizzotti} and RNE_{Pacheco} were plotted relative to N intake (Figure 4.5). Both RNEs presented strong correlations ($R^2>0.85$) with exponential equations as follows: $RNE_{Chizzotti} = -3.1865 + 0.6294 * \ln(N \text{ intake}(g/d))$ and $RNE_{Pacheco} = -1.902 + 0.4189 * \ln(N \text{ intake}(g/d))$.

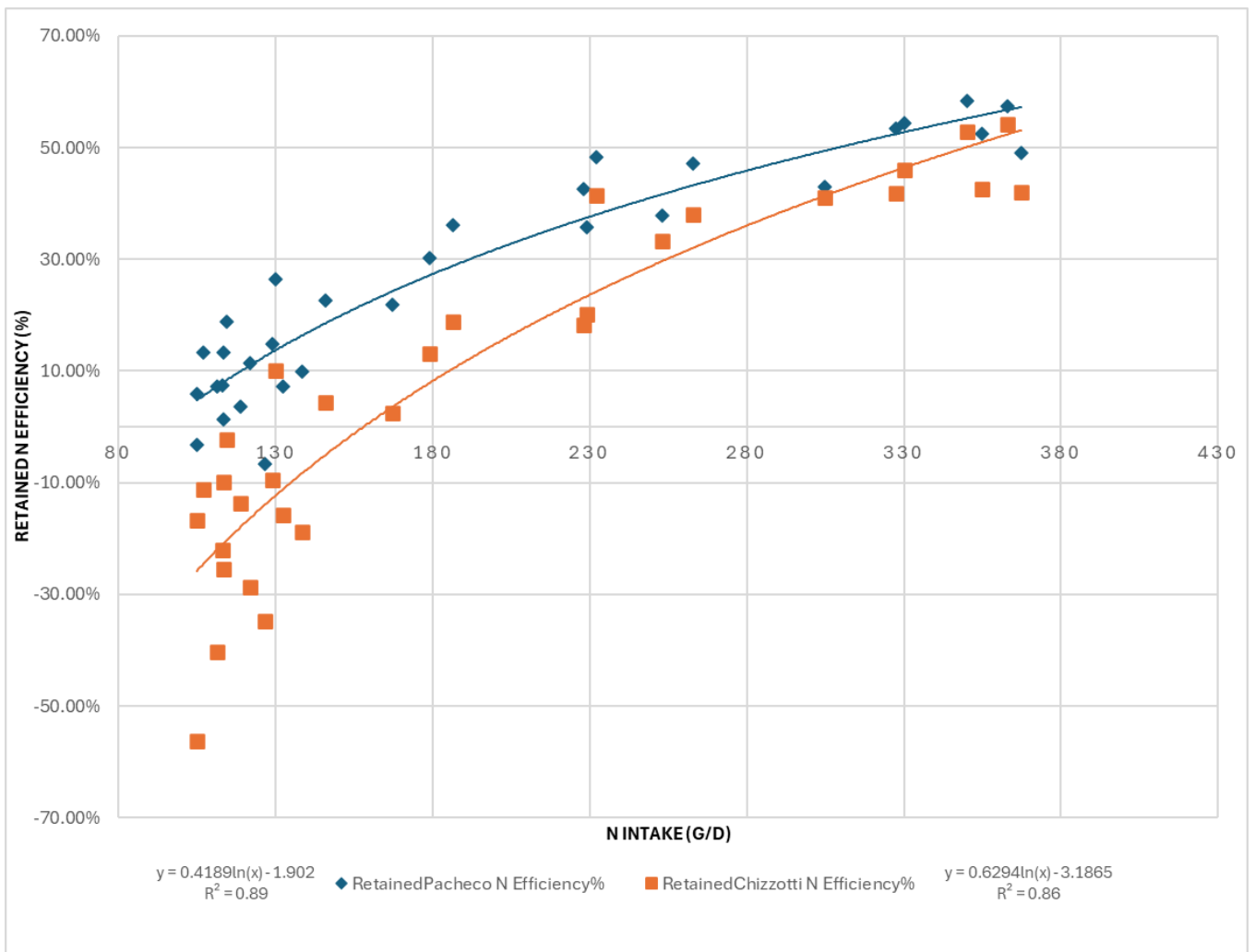


Figure 4.5: Retained N efficiency (RNE; %) relative to N intake (g/d) for rising one-year-old Hereford heifers fed lucerne silage. RNE values are grouped by urine nitrogen excretion (g/d) values used, giving $RNE_{Chizzotti}$ (%; ■) and $RNE_{Pacheco}$ (%; ◆), $R^2=0.86$, and $R^2=0.89$, respectively.

Chapter 5

Discussion

5.1 $\Delta^{15}\text{N}$

The positive, moderate correlation observed between $\Delta^{15}\text{N}$ and RNE in this study contradicts previously published literature (Cheng et al., 2011, 2013, 2014). Wheadon et al. (2014) reported a negative correlation between the N use efficiency of beef cattle and $\Delta^{15}\text{N}$, suggesting that under grazing conditions, N use efficiency declined as $\Delta^{15}\text{N}$ increased. In contrast, this study showed that RNE increased with increasing $\Delta^{15}\text{N}$. The positive relationship shown here is likely due to animals being presented as underfed, based on the estimated DMI derived from back-calculations. This speculation is supported by the positive relationship between N intake and RNE (Figure 4.5), where RNE increased exponentially as N intake increased. Inadequately fed animals typically exhibit negative energy balance and loss of LW (Swanson & Miller, 2008). However, the average LWG in this study was positive, suggesting that the majority of animals were fed above maintenance and gained weight. Thus, estimating DMI using ME requirements of heifers may have underestimated DM and subsequent N intake, producing an apparent positive response in RNE as N intake increased, and therefore a positive association with $\Delta^{15}\text{N}$. DM intake in this study was estimated based on equations from Rattray et al. (2017) for grazing beef cattle; however, these animals were not grazing and fed lucerne silage in bunkers. Whether this contributed to the underestimation of individual DMI is unclear. N balance studies in which DMI is directly measured are required to confirm the relationship between $\Delta^{15}\text{N}$ and RUE.

One additional factor that may explain the differing direction of the relationship between $\Delta^{15}\text{N}$ and RNE in this study compared with Cheng et al. (2011, 2013) is the study duration. In this study, LWG was measured over a two-week period, which is considerably shorter than in similar studies such as Wheadon et al. (2014), where LWG was measured over 12 weeks. Measuring LWG of ruminants over short periods, such as that used in this study, is associated with substantial error due to factors including variation in rumen fill. Because LWG was a major component used to calculate energy for production, this may have contributed to the inaccurate estimation of both N intake and LWG. These compounding errors likely resulted in an inaccurate estimation of RNE.

The $\Delta^{15}\text{N}$ was positively correlated with the digestible N (g/d) of an animal. This aligns with Cantalapiedra-Hijar et al. (2015), who presented a similar trend of increased $\Delta^{15}\text{N}$ values with increased N intakes and subsequent digestible N. An increased N intake increases ammonia production within the rumen during protein degradation. This ammonia is removed from the rumen

and taken to the liver for deamination, forming urea, which then re-enters the bloodstream. Therefore, increasing the N intake of an animal increases $\Delta^{15}\text{N}$ values by increasing blood urea N. The observed correlation is in opposition to Cheng et al. (2016), who reported that dietary N content did not affect $\Delta^{15}\text{N}$ when fed a similar dietary N content (4.1%). However, this was conducted in goats, and whether the physiology of N metabolism is different between goats and beef cattle is unclear. Further studies may be required to confirm the relationship between N intake, digestible N, and $\Delta^{15}\text{N}$ in ruminants fed different levels of dietary N.

5.2 Plasma Urea Nitrogen

The positive, moderate correlation observed between PUN and urinary N concentration (g/l) aligns well with previously reported literature (Burgos et al., 2007; Kohn et al., 2005; Kume et al., 2008). Kume et al. (2008) reported a positive correlation between PUN and urinary N concentration, suggesting that PUN could be used as a biological marker to estimate urine N. The increasing PUN values, causing increased urinary N, are a function of urea being filtered from the bloodstream into the urine. The filtration rate is controlled largely by the renal fluid clearance rate, which is the rate at which substances are filtered out of the kidneys (Lavery & Ferris, 2021). With higher PUN values, more urea is in the bloodstream and is then filtered out into the urine via the kidney. This raises the urinary N concentration, giving a positive correlation. Therefore, PUN can be used as a biomarker for predicting urinary N excretion in grazing cattle.

Improved N digestibility reduces N-digestion by-products, such as urea, in the circulatory system of ruminants (Chumpawadee et al., 2005). In this study, a negative correlation ($r=-0.49$) was observed between PUN and N digestibility (%). Similarly, Chumpawadee et al. (2005) reported lower PUN values when overall diet N digestibility increased through rumen synchrony (i.e. synchronising energy and crude protein supply in the rumen). When sufficient energy is available for rumen microbes, feed N digestibility can increase and PUN can decrease because microbes utilise ruminal ammonia more efficiently before it diffuses into the bloodstream and is converted to urea by the liver (Sinclair et al., 1993; Repetto et al., 2003). The negative relationship between PUN and N digestibility in our study was supported by the negative correlation ($r=0.47$; $P=0.008$) between urine urea and N digestibility, confirming the association between N digestibility and urea dynamics in ruminant body fluids. These results suggest PUN as a viable indicator of feed N digestibility in ruminants.

PUN, while having accurate correlations, has multiple caveats to ensure the validity of being a biomarker. The large diurnal variation of PUN must be accounted for when collecting samples; this variation is determined by diet and time since last feed (Kauffman & St-Pierre, 2001). Therefore, repeated measurements pre- and post-feed are required to form accurate predictions using PUN. Also, the collection method is invasive and impractical for large-scale implementation; therefore,

PUN currently resides in a niche of smaller-scale trials or trials with a large amount of funding and labour at its disposal.

5.3 N Intake

Quantifying an animal's N intake is essential for predicting associated N excretion in ruminants (Castillo et al., 2000). However, measuring individual N intake within a grazing system is practically impossible and thus indirect methods must be used. N intake forms an exponential relationship with RNE, where increasing N intake beyond a threshold increases the rate of N partitioned to urine and faeces (Beltran et al., 2022; Castillo et al., 2000; Shen et al., 2023). For example, Castillo et al. (2000) reported an exponential increase in urinary N excretion in dairy cows beyond a threshold N intake of 400 g/day, suggesting minimal N is directed to milk and a decline in N use efficiency at N intake levels higher than 400 g/d. Similarly, in our study, an exponential relationship was observed between N intake and RNE, where the rate of increase in RNE in response to increased N intake declined at higher levels of N intake. On the other hand, faecal N excretion (g/d) increased linearly, and urinary N excretion tended to increase with the increasing level of N intake. More beef cattle research is required to quantify feed quality (Feed ME and N content, as well as ME to N ratio) that optimise RNE but minimise N excreted to the environment.

5.4 Creatinine

A negative, strong correlation between urinary creatinine concentration (g/l) and urine volume was observed, which aligns with published literature (Chizzotti et al., 2008; David et al., 2015; Santos et al., 2017). Chizzotti et al. (2008) established a strong negative correlation between urinary creatinine concentration and urine volume; using this, the total urine volume can be calculated via creatinine spot sampling. Contrarily, strong positive correlations were observed between urinary creatinine excretion (g/l) and both urine N and urea concentrations (g/l). Moderate negative correlations were also found between creatinine and urinary N excretions (g/d) measured by both equations (Pacheco et al., 2009 and Chizzotti et al., 2008). Creatinine is produced at a relatively constant rate from muscle metabolism and is excreted unchanged in urine, making it a useful internal marker for estimating urine output in ruminants (Albin & Clanton, 1966). Because its excretion rate is stable and largely unaffected by diet, creatinine concentration can be used to adjust urinary N measurements, improving the accuracy of estimated daily N excretion. Our results confirm the wide use of creatinine as a good biomarker when total urine collection is not feasible.

5.5 Urine N to Creatinine ratio

A positive, moderate correlation was observed between UNCR and urine volume, which aligns with published literature (Moen & Delgiudice, 1997). UNCR (g/g) is a measure of protein synthesis within

the body, and Moen & Delgiudice (1997) present a positive correlation between urinary N excretion (g/d) and UNCR. Increasing dietary N intake increases protein synthesis, which increases PUN and subsequently daily urine N excretion (Albin & Clanton, 1966; Lavery & Ferris, 2021). With creatinine excretion being relatively stable irrespective of diet, increasing urine N content raises UNCR. This aligns with the positive moderate correlations of UNCR with urinary N excretion (g/d) and urine volume (l/d) found in the study (Table 4.3). UNCR is a reliable biomarker, with little diurnal variation observed relative to sampling time, making it more stable when compared to other biomarkers, for example, PUN (Albin & Clanton, 1966; Kauffman & St-Pierre, 2001).

5.6 Overview

To summarise, $\Delta^{15}\text{N}$ moderately correlated with RNE, digestible N (g/d), and retained N (g/d; Table 4.3). While these have been established previously in the literature (Aizimu et al., 2021; Cheng et al., 2013, 2014, 2016; Wheadon et al., 2014), correlations direction contrast previously published results. This is likely due to a short LWG measurement period and compounding uncertainties in back calculations, producing inaccurate data estimations.

While PUN is shown as a good biological marker for N metabolism, it has a large diurnal variation, and thus this must be accounted for when collecting samples, increasing the labour requirement for sample collection and limiting scale (Kauffman & St-Pierre, 2001).

N intake is a major determinant for urinary N excretion in ruminants; however, it is impractical to measure in large-scale grazing systems, thus indirect methods must be used (Beltran et al., 2022; Castillo et al., 2000). Plotting N intake data relative to RNE indicates that animals were underfed during the trial. However, this could potentially be attributed to the exacerbation of error thresholds within back-calculation estimations, which caused intakes to be artificially reduced.

Creatinine presented as a reliable marker for predicting ruminant urine output, through its stable excretion, unaffected by diet (Albin & Clanton, 1966).

UNCR observed strong validity in predicting daily urinary N excretion and is a good biological marker in place of solely creatinine when diet needs accounting for (Albin & Clanton, 1966; Moen & Delgiudice, 1997). UNCR possesses little diurnal variation, reducing the labour intensity of collection.

Chapter 6

Conclusion

Strong positive correlations were observed between N intake (g/d) and faecal N (g/d) excretion, N intake and retained N (g/d), and N intake and RNE. A tendency to positively correlate with urinary N (g/d) excretion was also observed. However, the impracticality of quantifying N intake within a large-scale grazing system necessitates the use of biomarkers.

Among the biomarkers investigated in this study, PUN showed a good correlation with RNE and urinary N (g/l). However, PUN exhibits large diurnal variation with feeding, which necessitates accounting for it when collecting samples.

$\Delta^{15}\text{N}$ is a more stable biomarker and showed a good correlation with RNE and digestible N intake (g/d); however, the direction of correlation was opposite to the literature. This highlights the limitations in estimating dry matter intake via back-calculations when using a small LWG period. This is further demonstrated by animals presented as underfed within the data.

Another biomarker is urine creatinine excretion (g/l), which results confirmed the efficacy for predicting ruminant urine output. UNCR also presented as a viable biomarker when diet is to be accounted for. Both Creatinine and UNCR possess little diurnal variation, giving reliable correlations for predictions to be based around.

These biomarkers present as viable for implementation into systems for indirectly quantifying nitrogen partitioning in grazing ruminants. Future studies should utilise LWG periods of increased duration to minimise error developed through intake back-calculation estimations.

References

- Adjabui, J. A., Morel, P. H. C., Morris, S. T., Kenyon, P. R., & Tozer, P. R. (2025). A comparison of three nutritional models for estimating total metabolisable energy requirements for a ewe, beef breeding cow, lamb, and a calf/yearling in New Zealand's pasture-only system. *Livestock Science*, 299. <https://doi.org/10.1016/J.LIVSCI.2025.105766>
- Aizimu, W., Al-Marashdeh, O., Hodge, S., Dewhurst, R. J., Chen, A., Zhao, G., Talukder, S., Edwards, G. R., & Cheng, L. (2021). Estimation of Nitrogen Use Efficiency for Ryegrass-Fed Dairy Cows: Model Development Using Diet- and Animal-Based Proxy Measures. *Dairy 2021, Vol. 2, Pages 435-451*, 2(3), 435–451. <https://doi.org/10.3390/DAIRY2030035>
- Albin, R. C., & Clanton, D. C. (1966). Factors Contributing to the Variation in Urinary Creatinine and Creatinine-Nitrogen Ratios in Beef Cattle. *Journal of Animal Science*, 25(1), 107–112. <https://doi.org/10.2527/JAS1966.251107X>
- Al-Marashdeh, O., Herath, H. M. G. P., Lee, Y. V., Tanner, J., & Beatson, P. R. (2025). Hereford heifers with lower blood urea nitrogen breeding values excrete urine with lower nitrogen concentration. *New Zealand Journal of Agricultural Research*, 68(4), 748–758. <https://doi.org/10.1080/00288233.2024.2398147>
- Ball, R., Keeney, D. R., Thoebald, P. W., & Nes, P. (1979). Nitrogen Balance in Urine-affected Areas of a New Zealand Pasture1. *Agronomy Journal*, 71(2), 309–314. <https://doi.org/10.2134/AGRONJ1979.00021962007100020022X>
- Beltran, I. E., Calvache, I., Cofre, R., Salazar, F., Keim, J. P., Morales, A., Pulido, R. G., & Alfaro, M. (2022). Nitrogen intake and its partition on urine, dung and products of dairy and beef cattle in Chile. *Agronomy*, 12(1), 15. <https://doi.org/10.3390/AGRONOMY12010015/S1>
- Beukes, P. C., Romera, A. J., Gregorini, P., Macdonald, K. A., Glassey, C. B., & Shepherd, M. A. (2017). The performance of an efficient dairy system using a combination of nitrogen leaching mitigation strategies in a variable climate. *Science of The Total Environment*, 599–600, 1791–1801. <https://doi.org/10.1016/J.SCITOTENV.2017.05.104>
- Cantalapiedra-Hijar, G., Fouillet, H., Huneau, J. F., Fanchone, A., Doreau, M., Nozière, P., & Ortigues-Marty, I. (2015). Relationship between efficiency of nitrogen utilization and isotopic nitrogen fractionation in dairy cows: Contribution of digestion v. metabolism? *Animal*, 10(2), 221–229. <https://doi.org/10.1017/S1751731115002025>
- Castillo, A. R., Kebreab, E., Beever, D. E., & France, J. (2000). A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution*. *Journal of Animal and Feed Sciences*, 9, 1–32.
- Cheng, L., Edwards, G. R., Dewhurst, R. J., Nicol, A. M., & Pacheco, D. (2016). The effect of dietary water soluble carbohydrate to nitrogen ratio on nitrogen partitioning and isotopic fractionation of lactating goats offered a high-nitrogen diet. *Animal*, 10(5), 779–785. <https://doi.org/10.1017/S1751731115002335>
- Cheng, L., Kim, E. J., Merry, R. J., & Dewhurst, R. J. (2011). Nitrogen partitioning and isotopic fractionation in dairy cows consuming diets based on a range of contrasting forages. *Journal of Dairy Science*, 94(4), 2031–2041. <https://doi.org/10.3168/jds.2010-3763>
- Cheng, L., Sheahan, A. J., Gibbs, S. J., Rius, A. G., Kay, J. K., Meier, S., Edwards, G. R., Dewhurst, R. J., & Roche, J. R. (2013). Technical note: Nitrogen isotopic fractionation can be used to predict nitrogen-use efficiency in dairy cows fed temperate pasture. *Journal of Animal Science*, 91(12), 5785–5788. <https://doi.org/10.2527/JAS.2012-5378>
- Cheng, L., Woodward, S. L., Dewhurst, R. J., Zhou, H., & Edwards, G. R. (2014). Nitrogen partitioning, energy use efficiency and isotopic fractionation measurements from cows differing in genetic merit fed low-quality pasture in late lactation. *Animal Production Science*, 54(10), 1651–1656. <https://doi.org/10.1071/AN14171>
- Chevront, S. N., Kenefick, R. W., Heavens, K. R., & Spitz, M. G. (2014). A comparison of whole blood and plasma osmolality and osmolarity. *Journal of Clinical Laboratory Analysis*, 28(5), 368–373. <https://doi.org/10.1002/JCLA.21695>

- Chizzotti, M. L., Valadares Filho, S. de C., Valadares, R. F. D., Chizzotti, F. H. M., & Tedeschi, L. O. (2008). Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. *Livestock Science*, *113*(2–3), 218–225. <https://doi.org/10.1016/J.LIVSCI.2007.03.013>
- Chumpawadee, S., Sommart, K., Vongpralub, T., & Pattarajinda, V. (2005). Effects of Synchronizing the Rate of Dietary Energy and Nitrogen Release on Ruminal Fermentation, Microbial Protein Synthesis, Blood Urea Nitrogen and Nutrient Digestibility in Beef Cattle. *Asian-Australasian Journal of Animal Sciences*, *19*(2), 181–188. <https://doi.org/10.5713/AJAS.2006.181>
- Cochran, R. C., Adams, D. C., Wallace, J. D., & Galyean, M. L. (1986). Predicting Digestibility of Different Diets with Internal Markers: Evaluation of Four Potential Markers. *Journal of Animal Science*, *63*(5), 1476–1483. <https://doi.org/10.2527/JAS1986.6351476X>
- David, D. B., Poli, C. H. E. C., Savian, J. V., Amaral, G. A., Azevedo, E. B., & Jochims, F. (2015). Urinary creatinine as a nutritional and urinary volume marker in sheep fed with tropical or temperate forages. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *67*(4), 1009–1015. <https://doi.org/10.1590/1678-4162-7759>
- Decau, M. L., Simon, J. C., & Jacquet, A. (2004). Nitrate Leaching under Grassland as Affected by Mineral Nitrogen Fertilization and Cattle Urine. *Journal of Environmental Quality*. <https://doi.org/10.2134/jeq2004.6370>
- Ellis, W. C., Poppi, D. P., Matis, J. H., Lippke, H., Hill, T. M., & Rouquette Jr, F. M. (1999). Dietary-digestive-metabolic interactions determining the nutritive potential of ruminant diets. *Nutritional Ecology of Herbivores*, 423–481.
- Fraser, B. C., Eyre, K., Gaughan, J. B., Wijffels, G., & Silva, L. F. P. (2025). Use of 15N abundance in tail hair to predict feed efficiency and response to a urea supplement in young cattle fed a tropical grass forage. *Animal Production Science*, *65*(6). <https://doi.org/10.1071/AN24210>
- Grafton, M. C. E., Bishop, P. A., & Bretherton, M. (2022). Using Monte Carlo Simulations to Achieve the Best Response From Nitrogen on Grazed Pasture Under a Legislated Nitrogen Cap in New Zealand: A Review. *Journal of the ASABE*, *65*(6), 1241–1247. <https://doi.org/10.13031/JA.15021>
- Gray, C. (2024). Nitrogen fertiliser use in grazed pasture-based systems in New Zealand: a summary. *New Zealand Journal of Agricultural Research*, *67*(6), 670–721. <https://doi.org/10.1080/00288233.2023.2198719>
- Gustafsson, A. H., & Palmquist, D. L. (1993). Diurnal Variation of Ruminal Ammonia, Serum Urea, and Milk Urea in Dairy Cows at High and Low Yields. *Journal of Dairy Science*, *76*(2), 475–484. [https://doi.org/10.3168/JDS.S0022-0302\(93\)77368-3](https://doi.org/10.3168/JDS.S0022-0302(93)77368-3)
- Herd, R. M., Oddy, V. H., & Richardson, E. C. (2004). Biological basis for variation in residual feed intake in beef cattle. 1. Review of potential mechanisms. *Australian Journal of Experimental Agriculture*, *44*(5), 423–430. <https://doi.org/10.1071/EA02220>
- Herremans, S., Decruyenaere, V., Cantalapiedra-Hijar, G., Beckers, Y., & Froidmont, E. (2020). Effects of hydrolysable tannin-treated grass silage on milk yield and composition, nitrogen partitioning and nitrogen isotopic discrimination in lactating dairy cows. *Animal*, *14*(4), 771–779. <https://doi.org/10.1017/S175173111900226X>
- Hoogendoorn, C. J., Lambert, M. G., Devantier, B. P., Theobald, P. W., & Park, Z. A. (2017). Nitrogen fertiliser application rates and nitrogen leaching in intensively managed sheep grazed hill country pastures in New Zealand. *New Zealand Journal of Agricultural Research*, *60*(2), 154–172. https://doi.org/10.1080/00288233.2017.1287100/ASSET/68272E73-DD07-4BFC-91B5-98DE89BD5BFA/ASSETS/IMAGES/TNZA_A_1287100_F0003_B.GIF
- Jonker, J. S., Kohn, R. A., & High, J. (2002). Use of Milk Urea Nitrogen to Improve Dairy Cow Diets. *Journal of Dairy Science*, *85*(4), 939–946. [https://doi.org/10.3168/JDS.S0022-0302\(02\)74152-0](https://doi.org/10.3168/JDS.S0022-0302(02)74152-0)
- Katongole, C. B., & Yan, T. (2020). Effect of Varying Dietary Crude Protein Level on Feed Intake, Nutrient Digestibility, Milk Production, and Nitrogen Use Efficiency by Lactating Holstein-Friesian Cows. *Animals 2020, Vol. 10, Page 2439, 10*(12), 2439. <https://doi.org/10.3390/ANI10122439>
- Kauffman, A. J., & St-Pierre, N. R. (2001). The Relationship of Milk Urea Nitrogen to Urine Nitrogen Excretion in Holstein and Jersey Cows. *Journal of Dairy Science*, *84*(10), 2284–2294. [https://doi.org/10.3168/JDS.S0022-0302\(01\)74675-9](https://doi.org/10.3168/JDS.S0022-0302(01)74675-9)

- Kohn, R. A., Dinneen, M. M., & Russek-Cohen, E. (2005). Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. *Journal of Animal Science*, 83(4), 879–889. <https://doi.org/10.2527/2005.834879X>
- Kolver, E. S., & Muller, L. D. (1998). Performance and Nutrient Intake of High Producing Holstein Cows Consuming Pasture or a Total Mixed Ration. *Journal of Dairy Science*, 81(5), 1403–1411. [https://doi.org/10.3168/JDS.S0022-0302\(98\)75704-2](https://doi.org/10.3168/JDS.S0022-0302(98)75704-2)
- Kume, S., Numata, K., Takeya, Y., Miyagawa, Y., Ikeda, S., Kitagawa, M., Nonaka, K., Oshita, T., & Kozakai, T. (2008). Evaluation of Urinary Nitrogen Excretion from Plasma Urea Nitrogen in Dry and Lactating Cows. *Asian-Australasian Journal of Animal Sciences*, 21(8), 1159–1163. <https://doi.org/10.5713/AJAS.2008.70734>
- Lavery, A., & Ferris, C. P. (2021). Proxy Measures and Novel Strategies for Estimating Nitrogen Utilisation Efficiency in Dairy Cattle. *Animals* 2021, Vol. 11, Page 343, 11(2), 343. <https://doi.org/10.3390/ANI11020343>
- Lee, C., & Hristov, A. N. (2013). Short communication: Evaluation of acid-insoluble ash and indigestible neutral detergent fiber as total-tract digestibility markers in dairy cows fed corn silage-based diets. *Journal of Dairy Science*, 96(8), 5295–5299. <https://doi.org/10.3168/JDS.2012-6442>
- Lund, P., Riis Weisbjerg, M., Hvelplund, T., Knud, & Knudsen, E. B., Weisbjerg, M. R., & Bach Knudsen, E. (2007). Determination of digestibility of different forages in dairy cows using indigestible NDF as marker. *Acta Agriculturae Scandinavica A: Animal Sciences*, 57(1), 16–29. <https://doi.org/10.1080/09064700701440421>
- MacLeod, C. J., & Moller, H. (2006). Intensification and diversification of New Zealand agriculture since 1960: An evaluation of current indicators of land use change. *Agriculture, Ecosystems & Environment*, 115(1–4), 201–218. <https://doi.org/10.1016/J.AGEE.2006.01.003>
- Matamura, M., & Kondo, M. (2025). Determination of urinary creatinine excretion and estimation of urinary nitrogen and purine derivatives in Wagyu, high-marbling fattening cattle. *Animal*, 19(7), 101576. <https://doi.org/10.1016/J.ANIMAL.2025.101576>
- Moen, R., & Delgiudice, G. D. (1997). Simulating Nitrogen Metabolism and Urinary Urea Nitrogen: Creatinine Ratios in Ruminants. *The Journal of Wildlife Management*, 61(3), 881. <https://doi.org/10.2307/3802197>
- Najem, O., Shah, M. M., Zubair, M., & Jesus, O. De. (2024). Serum Osmolality. *StatPearls [Internet]*. <https://www.ncbi.nlm.nih.gov/books/NBK567764/>
- Olmos Colmenero, J. J., & Broderick, G. A. (2006). Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. *Journal of Dairy Science*, 89(5), 1704–1712. [https://doi.org/10.3168/jds.s0022-0302\(06\)72238-x](https://doi.org/10.3168/jds.s0022-0302(06)72238-x)
- Orr, J. B. (1918). Creatine Excretion in Ruminants. *Biochemical Journal*, 12(3), 221. <https://doi.org/10.1042/BJO120221>
- Pacheco, D., Burke, J. L., Death, A. F., & Cosgrove, G. P. (2007, January). Comparison of models for estimation of urinary nitrogen excretion from dairy cows fed fresh forages. *Meeting the Challenges for Pasture-Based Dairying*.
- Parker, D. S., Lomax, M. A., Seal, C. J., & Wilton, J. C. (1995). Metabolic implications of ammonia production in the ruminant. *Proceedings of the Nutrition Society*, 54(2), 549–563. <https://doi.org/10.1079/PNS19950023>
- Pinares-Patiño, C. S., Waghorn, G. C., Hegarty, R. S., & Hoskin, S. O. (2009). Effects of intensification of pastoral farming on greenhouse gas emissions in New Zealand. *New Zealand Veterinary Journal*, 57(5), 252–261. <https://doi.org/10.1080/00480169.2009.58618>
- Powell, J. M., & Rotz, C. A. (2015). Measures of Nitrogen Use Efficiency and Nitrogen Loss from Dairy Production Systems. *Journal of Environmental Quality*, 44(2), 336–344. <https://doi.org/10.2134/JEQ2014.07.0299>
- Rattray, P. V., Brookes, I. M., & Nicol, A. M. (2017). *Pasture and Supplements for Grazing Animals* (14th ed.). New Zealand Society of Animal Production.
- Repetto, J. L., González, J., Cajarville, C., Alvir, M. R., & Rodríguez, C. A. (2003). Relationship between ruminal degradability and chemical composition of dehydrated lucerne. *Animal Research*, 52(1), 27–36. <https://doi.org/10.1051/ANIMRES:2003007>

- Rodríguez, R., Areadne, S., & Rodríguez, Y. (2007). Microbial protein synthesis in rumen and its importance to ruminants. *Cuban Journal of Agricultural Science*, 41(4), 287–294.
- Rumphorst, T., Scheu, T., Koch, C., & Sundrum, A. (2021). Inter- and Intra-Individual Variation in the Behavior of Feed Intake on Nutrient Availability in Early Lactating Dairy Cows. *Animals* 2022, Vol. 12, Page 37, 12(1), 37. <https://doi.org/10.3390/ANI12010037>
- Rys, G., Gibbs, J., Clark, D., Lambert, G., Clark, H., & Newman, M. (2021). Productivity, changes and resilience in New Zealand grassland agriculture over the last three decades. *NZGA: Research and Practice Series*, 17, 37–54. <https://doi.org/10.33584/RPS.17.2021.3461>
- Santos, S. A., Prates, L. L., Carvalho, G. G. P. de, Santos, A. C. S. dos, Valadares Filho, S. de C., Tosto, M. S. L., Mariz, L. D. S., Neri, F. da S., & Sampaio, M. de Q. (2017). Creatinine as a metabolic marker to estimate urinary volume in growing goats. *Small Ruminant Research*, 154, 105–109. <https://doi.org/10.1016/J.SMALLRUMRES.2017.08.007>
- Shen, C., Wang, J., Zhao, G., & Li, M. M. (2023). A meta-analysis of dietary metabolizable amino acids and energy supply on nitrogen retention and nitrogen utilization efficiency in beef cattle. *Animal Feed Science and Technology*, 302, 115670. <https://doi.org/10.1016/J.ANIFEEDSCI.2023.115670>
- Spanghero, M., & Kowalski, Z. M. (2021). Updating analysis of nitrogen balance experiments in dairy cows. *Journal of Dairy Science*, 104(7), 7725–7737. <https://doi.org/10.3168/JDS.2020-19656>
- Spek, J. W., Dijkstra, J., Van Duinkerken, G., & Bannink, A. (2013). A review of factors influencing milk urea concentration and its relationship with urinary urea excretion in lactating dairy cattle. *The Journal of Agricultural Science*, 151(3), 407–423. <https://doi.org/10.1017/S0021859612000561>
- Stats NZ. (2024). *Agricultural production statistics: Year to June 2024 (final) | Stats NZ*. <https://www.stats.govt.nz/information-releases/agricultural-production-statistics-year-to-june-2024-final/>
- StatsNZ. (2024). *Farm numbers and farm size: Data to 2022*.
- Sun, X., Luo, N., Longhurst, B., & Luo, J. (2008). Fertiliser Nitrogen and Factors Affecting Pasture Responses. *The Open Agriculture Journal*, 2(1), 35–42. <https://doi.org/10.2174/1874331500802010035>
- Swanson, K., & Miller, S. (2008). Factors regulating feed efficiency and nutrient utilization in beef cattle. *Mathematical Modelling in Animal Nutrition*, 419–441. <https://doi.org/10.1079/9781845933548.0419>
- Valizadeh, A., Kazemi-Bonchenari, M., Khodaei-Motlagh, M., Moradi, M. H., & Salem, A. Z. M. (2021). Effects of different rumen undegradable to rumen degradable protein ratios on performance, ruminal fermentation, urinary purine derivatives, and carcass characteristics of growing lambs fed a high wheat straw-based diet. *Small Ruminant Research*, 197, 106330. <https://doi.org/10.1016/J.SMALLRUMRES.2021.106330>
- Vérité, R., Delaby, L., Vérité, R., & Delaby, L. (2000). Relation between nutrition, performances and nitrogen excretion in dairy cows. *Annales de Zootechnie*, 49(3), 217–230. <https://doi.org/10.1051/ANIMRES:2000101>
- Waldrip, H. M., Todd, R. W., & Cole, N. A. (2013). Prediction of nitrogen excretion by beef cattle: A meta-analysis. *Journal of Animal Science*, 91(9), 4290–4302. <https://doi.org/10.2527/JAS.2012-5818>
- Wheadon, N. M., McGee, M., Edwards, G. R., & Dewhurst, R. J. (2014). Plasma nitrogen isotopic fractionation and feed efficiency in growing beef heifers. *British Journal of Nutrition*, 111(9), 1705–1711. <https://doi.org/10.1017/S0007114513004078>