

The effect of dietary nitrogen on nitrogen partitioning and milk production in grazing dairy cows

A thesis presented in partial fulfilment of the requirements for the Master of Animal Science at Massey University Palmerston North, New Zealand

Stacey Hendriks

2016

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

ABSTRACT

Two experiments were conducted during spring (8th October to 12th November 2009) as part of a larger study, to study the effects of increasing levels of crude protein (CP) in pasture on milk production, dry matter intake (DMI) and nitrogen (N) partitioning in dairy cows.

The first experiment was undertaken over 25 days (8th October to 1st November 2009), where fifteen multiparous, rumen fistulated, early lactation Holstein-Friesian cows (505 \pm 10.4 kg liveweight; 4.1 body condition score \pm 0.044, mean \pm standard deviation) were assigned to one of three urea supplementation treatments: Control (0 g/day urea; ~20% CP), Medium (350 g/day urea; ~25% CP) and High (690 g/day urea: ~30% CP). Urea was supplemented to the pasture-based diet to increase CP content while maintaining similar concentrations of all other nutrients across treatments. All cows were offered ~20 kg dry matter (DM)/day perennial ryegrass-based pasture (CP = 20.6 \pm 0.56% DM; metabolisable energy (ME) = 11.8 \pm 0.06 MJ/kg DM). Cows were acclimated to their urea treatment over a 25 day experimental period. The objective of this study was to determine the effect of increased dietary CP in grazing cows on DMI and milk yield.

Dry matter intake was estimated using a back calculation method from the energy requirements of the cows. The results indicate a complex interaction between DMI, milk yield and urea intake. As dietary CP increased, the milk yield increased; however, as urea's contribution to total dietary CP concentration increased, the increase in both DMI and milk yield was less. Milk yield decreased when urea supplementation increased beyond 350 g/day, and the interaction evident in milk yield was mirrored in yields of fat, CP and lactose (P <0.001). The addition of urea had no effect on milk fat, protein and lactose percentages.

The second experiment was conducted over 22 days (22^{nd} October to 12^{th} November 2009), involving ten multiparous, rumen fistulated, early lactation Holstein-Friesian cows (520 ± 5.6 kg liveweight; 4.15 body condition score \pm 0.078, mean \pm standard deviation). This experiment was undertaken to study N partitioning in pasture-fed grazing dairy cows using urea supplementation as a non-protein N (NPN) model to ensure all other nutritional characteristics of the forage remained the same. All cows were offered ~19 kg DM/day of perennial ryegrass-based pasture (CP = 18.4 \pm 0.64% DM; ME = 11.4 \pm 0.06 MJ/kg DM). Cows were assigned to one of two experimental groups: Control (0 g/day urea; ~18% CP), and a Urea supplemented group (350 g/day urea; ~23% CP). Cows were acclimated to the diets and metabolism stalls for 14 days, and a further 7 days were used for total collection of urine, faeces and milk.

Increasing dietary CP content had no effect on DMI, milk yield, milk composition, and faecal N. Urinary urea N (UUN) and urine N yield and concentrations increased as dietary CP content increased however, urinary creatinine, ammonia (NH₃), calcium and magnesium were not affected. Rumen urea and NH₃ concentrations were increased as CP content increased. Milk urea N showed trends for linear responses to increasing N intake (P <0.001, R² = 0.47). A 16.5% increase in N intake resulted in a 42.5% increase in milk urea nitrogen (MUN) concentration; however, the relationship was restricted to low MUN concentrations. Urinary N increased linearly as a result of N intake, although the relationship was restricted due to the underestimation of urinary N and the limited range of N intake values. The 28% increase in urinary N excretion resulted from a sharp 3.6% decline in N efficiency as dietary N content increased.

The main conclusions of this thesis were the ability for excessive urea intake to reduce milk yield in grazing dairy cows. Further research is needed to determine if high soluble NPN concentrations in fresh pasture would affect DMI and milk yield in the same way. Increasing N intake results in linear increases in MUN, urinary N and UUN. These relationships could provide useful tools to predict urinary N excretion due to the strong relationships between these variables. Further research is needed to develop robust prediction equations for the relationships between these variables in grazing dairy cows before they could be used as regulatory tools.

INTRODUCTION

Farming has played an important role in New Zealand's economy for over 100 years (PCE, 2004). Dairy and meat products are the single biggest export earners and currently comprise ~50% of New Zealand's export income (Statistics NZ, 2012). Overall, farming contributes over 5% of gross domestic product in New Zealand and is significantly important to the economy (Statistics NZ, 2012).

There are, currently, around 60,000 farms in New Zealand, with more than half of New Zealand's land area used for farming including production forestry (PCE, 2004; Statistics NZ, 2012). As illustrated in Figure 1, the dominant land use is sheep farming; however, dairy farming also makes up a significant portion of the land use and is the largest industry (~\$15.8 billion) in New Zealand, accounting for 25% of export income (Statistics NZ, 2012). The New Zealand dairy industry is the largest single contributor to internationally traded dairy products, with a trade value of nearly US\$ 8 billion (United Nations, 2014).



Figure 1: New Zealand land area distribution for different farming types in 2012; excluding forestry and 'other' usage, adapted from (Statistics NZ, 2012).*Viticulture is included within horticulture, as vineyards make up only about 0.4% of the total land area farmed in New Zealand. The total area of land used for farming is approximately 14 million hectares.

New Zealand's dairy farming has traditionally centred on a seasonal, low-cost pasture-based system,

where cows calve in late winter/early spring and are subsequently milked during spring, summer and

autumn, but 'dried off' (a management technique to cease lactation) during late autumn/winter when pasture growth is minimal (Ulyatt, 1997; PCE, 2004). This results in a large volume of milk available during spring/early summer. As a result, the global demand for milk products is a key driver of production in New Zealand due to the domestic market being too small to utilise the milk product available (Scarsbrook and Melland, 2015). The pasture-based system allows New Zealand to remain competitive on the international market due to the associated low cost of production (Ulyatt, 1997). Dairy farmers in New Zealand are paid in relation to the fat and protein supplied and the price paid is consistent throughout New Zealand. However, this price is dependent on world market prices, and is therefore difficult to predict (Penno and Kolver, 2000). Combinations of increasing global demand for milk products, along with ongoing financial pressure to increase efficiency both on farm and throughout the industry, have driven the intensification of dairying in New Zealand (PCE, 2004; Scarsbrook and Melland, 2015).

To illustrate this intensification, in 1993/94 there were 2.7 million cows on 1.1 million hectares in New Zealand and 20 years later in 2014/15 there were 5.0 million cows on 1.7 million hectares (LIC and DairyNZ, 2015). Along with this substantial increase in cow numbers and area under dairy farming in New Zealand, the milk yield per hectare increased due to both an increase in stocking rate and an increase in milk yield per cow (Table 1) (LIC and DairyNZ, 2015). As a result, the total milksolids yield increased 157% between 1994 and 2015 (Table 1).

Total	Industry estimate 2014-2015	% Change over time 1994-2015
Dairy cows, million	5.0	82%
Area under dairy, million ha	1.7	55%
Milk yield, million kg milksolids	1890	157%
Average		
Stocking rate, cows/ha	2.87	18%
Herd size, cows/farm	419	123%
Milk yield, kg milksolids/cow	377	36%
Milk yield, kg/ha	1082	53%

Table 1: Comparative dairy industry figures highlighting potential drivers of environmental impact.Information is sourced from LIC (1994) and LIC and DairyNZ (2015).

ha = hectares

This intensification is due to a large area of sheep, and beef farms and some forestry in the South Island being converted to dairy, due to the availability of water resources for irrigation (PCE, 2013). In 1994, the South Island contained 12% of the total dairy cattle population in New Zealand (LIC, 1994). This increased by 28% from 1994-2015, resulting in a total dairy cow population in the South Island of 2.0 million (LIC, 1994; LIC and DairyNZ, 2015). The current distribution of the New Zealand dairy herd by region is presented in Figure 2 (LIC and DairyNZ, 2015).



Figure 2: Regional distribution of dairy cows (percentage of total herd) in New Zealand (2014/15) (LIC and DairyNZ, 2015).

New Zealand's pasture-based system results in the feeding of pasture that often contains high concentrations of crude protein (CP) (Ulyatt, 1997), which, in excess, is not utilised efficiently by the dairy cow. These high concentrations of CP are a result of the timing of grazing and are further excacerbated by nitrogen (N) fertiliser inputs (Van Vuuren *et al.*, 1991; Lambert *et al.*, 2004). The growth and intensification of dairying in the past 20 years has resulted in a 17% increase in stocking

rate (Table 1) and an almost 7-fold increase in the use of N fertiliser (from 75,800 t in 1993 to 511,074 t in 2013) (Statistics NZ, 2012).

As a result of intensification of dairy farming in New Zealand, water quality has declined due to increased nutrient loading and subsequent eutrophication (Ballantine and Davies-Colley, 2014). Urine from farm animals is the major source of N in New Zealand's waterways draining agricultural catchments (PCE, 2013;). Over a 22 year time series (1990-2011), excreted N loads from dairy cows has more than doubled (102% increase) and nitrate (NO₃⁻) concentrations in waterways have also increased (Figure 3) (Scarsbrook and Melland, 2015). Increased N fertiliser use has also resulted in increased dietary N intake and subsequent deposition of urinary N on pasture, due to inefficient N use by the dairy cow as well as smaller losses of N fertiliser (Monaghan *et al.,* 2005).



Figure 3: National trends in nitrogen excreted load to land (t/year) from all stock types for the period 1990-2011 in New Zealand (Scarsbrook and Melland, 2015).

The loss of NO₃⁻ due to its susceptibility to leaching from soil into groundwater is of major concern to freshwater ecosystems. Urinary N enters groundwater via drainage through soil and N fertiliser enters surface waterways via runoff or direct input (Clark, 1997). Together these have adverse effects on water quality due to accelerated eutrophication of surface water, resulting in the increased growth of algae and nuisance weeds, causing a shortage of oxygen for aquatic life (Gregg *et al.,* 1993). This can result in death of aquatic organisms, can have adverse effects on tourism, and increase treatment costs, for potable water (Di and Cameron, 2005; MFE, 2014).

The New Zealand dairy industry is striving for increased productivity, whilst maintaining or reducing the environmental footprint, with particular emphasis on reducing N leaching to waterways (Pacheco *et al.*, 2007). Consequently, research into the reduction of N losses from dairy farm systems is a high priority for the dairy industry to comply with environmental standards (DairyNZ, 2014). Animal nutrition is a major management tool to reduce the N lost through urine; however, a reduction in dietary N can also negatively affect animal production (Fanchone *et al.*, 2013). Therefore, it is important to understand N partitioning in the cow to provide guidelines and models to mitigate N losses through managing nutrition (Tamminga, 1992).

ABBREVIATIONS

ADF	Acid detergent fibre
АТР	Adenosine triphosphate
СР	Crude protein
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
H⁺	Hydrogen ion
ME	Metabolisable energy
MJ ME	Megajoules metabolisable energy
MP	Microbial protein
MUN	Milk urea nitrogen
Ν	Nitrogen
NDF	Neutral detergent fibre
NIRS	Near-infrared spectroscopy
NPS-FM	National Policy Statement for Freshwater Management
N ₂ O	Nitrous oxide
NO ₃ ⁻	Nitrate
NH ₃	Ammonia
NH_4^+	Ammonium
NPN	Non-protein nitrogen
ОМ	Organic matter
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
SP	Soluble protein
TLI	Trophic level index
UUN	Urinary urea nitrogen
WSC	Water-soluble carbohydrates

ACKNOWLEDGEMENTS

Firstly, I would like to thank my two supervisors, Prof. Danny Donaghy and Dr. John Roche. I am incredibly grateful that I have had two incredibly helpful and supportive supervisors to guide me through my Master's degree. I could not have completed my thesis to a high standard without your input.

Danny, thank you for your mentoring during my university journey as both an undergraduate and post-graduate student. Thank you for always having a listening ear; that I could always come and discuss my ideas, progress and when needed, general life matters. Thank you for always accommodating my study within your busy work schedule. Thank you for reading countless drafts and giving me invaluable and occasionally comical feedback with the writing of this thesis.

John, your enthusiasm about research science and particularly animal nutrition was a major catalyst that encouraged me to enrol as a post-graduate student. Thank you for your prompt replies to my countless emails no matter where you were in the world, and taking time out of your busy schedule to fit in meetings. Thank you for your invaluable knowledge and feedback that contributed to the completion of my thesis.

In addition, I would like to thank the rest of my thesis committee. Dr. Angela Sheahan thanks for your insightful comments and help with the collection of my data. Prof. Nicolas Lopez-Villalobos thanks for your statistical advice and encouragement.

Thank you DairyNZ Inc and MPI for funding the experimental programme through the Sustainable Farming Fund. Thank you Massey University for assisting me in the completion of my Master's thesis. I also want to thank my fellow IVABS post-graduate friends for the stimulating discussions, and the much needed laughs during stressful times throughout completing my Master's degree.

Finally, I would like to thank my family, friends and partner. Thank you for your love and support. Particular thanks to my parents who have supported and encouraged me throughout my university endeavours and my life. A big thanks and lots of love go to my partner, Jack, for all his love, wise words and support during the last 2 years.

LIST OF FIGURES

Figure 1: New Zealand land area distribution for different farming types in 2012; excluding forestry and 'other' usage, adapted from (Statistics NZ, 2012).*Viticulture is included within horticulture, as vineyards make up only about 0.4% of the total land area farmed in New Zealand. The total area of land used for farming is approximately 14 million hectares
Figure 2: Regional distribution of dairy cows (percentage of total herd) in New Zealand (2014/15) (LIC and DairyNZ, 2015)v
Figure 3: National trends in nitrogen excreted load to land (t/year) from all stock types for the period 1990-2011 in New Zealand (Scarsbrook and Melland, 2015) vi
Figure 4: Cow density at district scale. Stars indicate areas where existing dairy farmers are operating under regulated nitrogen limits (Scarsbrook and Melland, 2015)
Figure 5: Transformations of nitrogen in grazed legume based pasture (R; complex organic molecule, N ₂ ; nitrogen gas, N ₂ O; nitrous oxide, NO; nitric oxide, NH ₃ ; ammonia gas, NH ₄ ⁺ ; ammonium ion, NO ₂ ⁻ ; nitrite and NO ₃ ⁻ ; nitrate)
Figure 6: Digestion and metabolism of nitrogenous compounds in the rumen (McDonald <i>et al.,</i> 2011)
Figure 7: Dry matter intake (DMI) response (kg/day) to dietary crude protein (% CP) and urea (U, % of CP) in the diet (Polan <i>et al.</i> , 1976)15
Figure 8: Milk yield response (kg/day) to dietary crude protein (% CP) and urea (U, % of CP) in the diet (Polan <i>et al.</i> , 1976)
Figure 9: Milk yield (kg/day) versus dry matter intake (DMI) (kg/day) with varying dietary crude protein (% CP) and urea (U) (% of CP) in the diet (Polan <i>et al.,</i> 1976)16
Figure 10: Total daily urea production (diamonds) and gut urea entry rate (squares) relative to nitrogen (N) intake in growing cattle (solid symbols) and lactating dairy cows (open symbols), as measured using dual-labeled urea infusions (adapted from Reynolds and Kristensen, 2008).
Figure 11: Dietary crude protein (CP) concentration and the fraction of total urea production (UER) that is returned to the gut via blood and saliva (A) or excreted in urine and milk (B) in cattle, as measured using dual-labeled urea infusions (adapted from Reynolds and Kristensen, 2008)19
Figure 12: Interplay of dietary, ruminal, and extraruminal sources of nitrogen and amino acids (AA). CH ₂ O = carbohydrates; Export - milk, conceptus, scurf, secretions. Gut or tissue compartments are labelled in bold, italic print (Hall and Huntington, 2008)
Figure 13: Relationship between total nitrogen (N) intake (g/day) and faecal, milk and urinary N outputs (g/day). The fitted lines were given by equations 1 (), 2 () and 3 () (Castillo <i>et al.</i> , 2000)

Figure 14: Relationship between total nitrogen intake (g/day) and faecal, milk and urinary nitrogen outputs (g/day). The fitted lines were given by equations 4, 5 and 6 in Table 5 (Kebreab <i>et al.</i> , 2001)
Figure 15: Relationship between total nitrogen (N) intake (g/day) and faecal (circles), milk (triangles) and urinary (squares) N outputs (g/day). The fitted lines were according to equations 7, 8 and 9 in Table 6 (Kebreab <i>et al.,</i> 2001)
Figure 16: Effect of type of energy source on faecal (solid), urinary (unshaded) and milk (grey) nitrogen (g/day) (Kebreab <i>et al.,</i> 2001)
Figure 17: Effect of protein degradability on faecal (solid), urinary (unshaded) and milk (grey) nitrogen (g/day) (Kebreab <i>et al.,</i> 2001)27
Figure 18: Perennial ryegrass herbage changes in crude protein (g/kg dry matter (DM)), non- structural carbohydrates (g/kg DM), metabolisable energy (MJ ME/kg DM) and neutral detergent fibre (g/kg DM) with regrowth (Chaves <i>et al.</i> , 2006)
Figure 19: Mean urea intake (g/day) in the High (circles) and Medium (squares) treatments during the 5 periods over the course of the experiment
Figure 20: Diurnal profile of cows grazing during peak lactation. Shading represents milking time and vertical dashed lines represent sunrise and sunset. Green arrows represent additions of urea. Modified from Sheahan <i>et al.</i> (2011)
Figure 21: Interaction between urea treatment and period in estimated dry matter intake (DMI), dietary crude protein content (CP %), milk yield and the yield of milk components (P <0.001); Control (open circles), Medium (squares) and High (circles)
Figure 22: Relationship between urea intake (g/day) and milk yield (kg/day) for all cows (y = 20.5 + 0.0029x, x ≤ c; y = 20.5 + c (350 (0.0029 - 0.0235)) + 0.0235x, x > c; c = 350 g urea/day; P <0.001)
Figure 23: Relationship between crude protein content (CP %) and milk yield (kg/day) for all cows (y= 36.175 - 0.612x; P < 0.001; R ² = 0.45)
Figure 24: Relationship between milk yield (kg/day), dry matter intake (DMI) (kg/day) and urea intake (g/day) (y = -21.05 + 2.69d + 0.028x - 0.0012d ¹ - 0.00000115d ² ; R ² = 0.75; P < 0.001)51
Figure 25: Relationship between total urine urea nitrogen (mmol/d) and total nitrogen intake (g/d) for all cows
Figure 26: Relationships between total urine nitrogen (g/day), estimated urine nitrogen (g/day) and total nitrogen intake (g/day) for all cows
Figure 27: Relationships between milk urea nitrogen (mmol/L) and total nitrogen intake (g/day) and total urine nitrogen (g/day) for all cows

Figure 28: Relationship between milk urea nitrogen (mmol/L) and rumen ammonia (NH ₃ -N)	
concentrations (mg/dL) for all cows	68

Figure 29: Comparison of the predictions of urinary nitrogen excretion (g/day) based on milk urea nitrogen (mg/dL) according to the current study (y = 75.6 + 17.8x; R² = 0.32), along with studies by Jonker *et al.* (1998), Kauffman and St-Pierre (2001) and Nousiainen *et al.* (2004). .76

LIST OF TABLES

Table 1: Comparative dairy industry figures highlighting potential drivers of environmental impact.
Information is sourced from LIC (1994) and LIC and DairyNZ (2013)iv
Table 2: Crude protein concentration (CP %), nitrogen (N) fractions (g/kg dry matter (DM))
degradation rate of true protein (k) and rumon undegradable protein digestibility (PLID %) of
degradation rate of true protein (k_d) and rumen undegradable protein digestibility $(KOP \%)$ of
grass – legume mix and total mixed ration (TMR) diets (adapted from NRC, 2001)
Table 3: Effects of supplementing different carbohydrate types to grazing dairy cows in early
lactation on productive nitrogen (N) output, milk urea nitrogen (MUN), blood urea nitrogen
(BUN) and urinary N to creatinine (N:creatinine) (Higgs <i>et al.,</i> 2013)20
Table 4: Intake, nitrogen (N) partitioning and milk yield by cows fed the synchronous diet (SYND) or
the asynchronous diet (ASYND) (Kolver <i>et al.,</i> 1998)22
Table 5: Relationship between nitrogen intake (N_i) , faecal nitrogen (N_f) , milk nitrogen (N_m) and
urinary nitrogen (N _u) across two studies (Castillo <i>et al.,</i> 2000; Kebreab <i>et al.,</i> 2001)23
Table 6: Relationship between nitrogen intake (N_i) , faecal nitrogen (N_f) , milk nitrogen (N_m) and
urinary nitrogen (N _u) for diets of similar composition (Kebreab <i>et al.,</i> 2001)
Table 7: Nutrient composition of experimental diets and means for nitrogen intake (NI), net energy
(NE), milk urea nitrogen (MUN), blood urea nitrogen (BUN) and urine urea nitrogen (UUN)
excretion (Burgos <i>et al.,</i> 2007)28
Table 8: Dietary crude protein (CP %), dry matter intake (DMI) and nitrogen (N) intake of dairy cows
offered increasing amounts of urea as a supplement to pasture during early lactation in the
Control Medium and High treatments 46
Table 9: Yield of milk (kg/day) and milk components of dairy cows offered increasing amounts of
urea as a supplement to pasture during early lactation in the Control, Medium and High
treatments48
Table 10: Sequence of equations used to determine final models of milk yield (kg/day). 50
Table 11: Chemical composition of pasture fed to all treatments (mean ± standard error). Percentage
crude protein (% CP), available protein (% AP), soluble protein (% SP), water soluble
carbohydrates (% WSC), acid detergent fibre (% ADF), neutral detergent fibre (% NDF), lignin,

dry matter (% DM), organic matter (% OM), organic matter digestibility (% OMD), dry matter digestibility (% DMD), and metabolisable energy (ME). Wet chemistry analysis (Dairy One, Analytical Services, New York, USA) and NIRS (NIRS; Feed Tech, Palmerston North, New Zealand).

Table 13: Intake, liveweight, digestibility of feed and faecal weight in the Control and Urea
supplement treatments. Dry matter intake (DMI), nitrogen (N), crude protein (CP) dry matter
(DM), and dry matter digestibility (DMD)62
Table 14: Water balance in the Control and Urea supplement treatments
Table 15: Yield of milk and milk components of the Control and Urea supplement treatments during
the 7 day nitrogen balance period63
Table 16: Yield of urine (mmol/d) and urine components of the Control and Urea supplement
treatments during the 7 day nitrogen balance period64
Table 17: Nitrogen parameters pertaining to partitioning in urine, faeces, milk and rumen fluid of
cows grazing pasture in the Control and Urea supplement treatments
Table 18: Urea dosing schedule for the Medium and High treatments during the experimental
period
Table 19: Urea dosing schedule for the Urea supplement treatment during the acclimation period. 89
Table 20: Urea dosing schedule for the Urea supplement treatment during the metabolism stall and
collection period
Table 21: Yield of milk (kg/day), milk components, pasture intake and pasture N content from dairy
cows offered increasing amount of urea as a supplement to pasture during early lactation90

ABSTRACTi
INTRODUCTIONiii
ABBREVIATIONS viii
ACKNOWLEDGEMENTSix
LIST OF FIGURES x
LIST OF TABLES xiii
Chapter 1: Review of literature; Nitrogen-use efficiency in dairy cows and its adverse effects on
the environment3
1.1 Introduction
1.2 Water quality in New Zealand3
1.3 The nitrogen cycle6
1.4 Nitrogen metabolism7
1.4.1 Protein degradation8
1.5 Ammonia toxicity 11
1.6 Supplementing feed grade urea13
1.7 Urea recycling
1.8 Nutrient synchrony19
1.9 Relationships between dietary nitrogen and urinary nitrogen23
1.9.1 Other factors influencing nitrogen-use efficiency25
1.10 Dietary nitrogen supply 29
1.11 Sources of error in N balance studies
1.12 Conclusion
Chapter 2: Effect of high-urea supplementation on pasture dry matter intake and milk production
in grazing dairy cows

TABLE OF CONTENTS

2.2.3 Milking and milk sampling	41
2.2.4 Liveweight and body condition score	41
2.2.5 Calculations and statistical analysis	42
2.2.5.1 Calculations	42
2.2.5.2 Statistical analysis	
2.3 Results	46
2.3.1 Dry matter intake and milk production	46
2.4 Discussion	52
Conclusion	53
Chapter 3: Effect of urea supplementation on N partitioning in dairy cows	54
3.1 Introduction	54
3.2 Materials and methods	56
3.2.1 Experimental design	
3.2.2 Pasture offered	
3.2.3 Milking and milk samples	
3.2.4 Liveweight and body condition score	
3.2.5 Urine and faecal sampling	59
3.2.6 Ruminal samples	60
3.2.7 Calculations and statistical analysis	60
3.2.7.1 Calculations	60
3.2.7.2 Statistical analysis	61
Chapter 3.3: Results	62
3.3.1 Intake, digestibility of feed, liveweights and faecal weight	62
3.3.2 Water balance	62
3.3.3 Milk production	63
3.3.4 Urine excretion	63
3.3.5 Nitrogen balance	64
Chapter 3.4: Discussion	69
Nitrogen intake	69
Faecal nitrogen	70
Milk production	70
Nitrogen balance	72

Urinary nitrogen	73
Milk urea nitrogen	74
Ruminal nitrogen	77
Conclusion	79
General discussion and conclusions	
APPENDICES	
Appendix 1: Analytical procedures adapted from (Dairy One, 2014)	
Dry matter	84
Protein	84
Crude protein	84
Soluble protein	84
Fibre	85
Acid detergent fibre (ADF)	85
Neutral detergent fibre (NDF)	85
Lignin	86
Minerals	86
Supplemental services	87
Ash	87
Starch	87
Appendix 2: Urea dosing schedule for Chapter 2	
Appendix 3: Urea dosing schedules for Chapter 3	
Appendix 4: Additional data for Chapter 2	90
BIBLIOGRAPHY	91