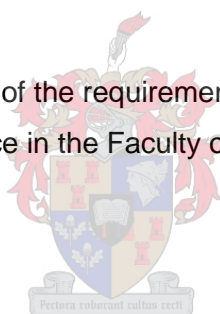# Forage fibre quality as a determinant of nitrogen use efficiency in dairy cows

by

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Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Animal Science in the Faculty of AgriSciences

At

Stellenbosch University

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Date: March 2017

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

March 2017

Abstract

Pollution from dairy farming, in the form of nitrogen emissions, is becoming an increasing concern globally due to greenhouse gasses and global warming. Many countries around the world, especially in the European Union (EU), have already put in place regulations regarding treatment of dairy farm effluent in an attempt to reduce nitrogen emissions. Many studies are also focussing on possible ways in which nitrogen excretion from cows can be reduced. Although none of these regulations are currently in place in South Africa it is likely that they soon will be as our agricultural practices follow EU regulations closely.

Four lactating Holstein cows were used in a 4 x 4 Latin square design balanced for carryover effects with a 2 x 2 factorial arrangement of treatment. Treatments were low crude protein (CP) concentration with high neutral detergent fibre (NDF) digestibility (**LpHd**), high CP concentration with high NDF digestibility (**HpHd**), low CP concentration with low NDF digestibility (**LpLd**) and high CP concentration with low NDF digestibility (**HpLd**). Crude protein concentrations for the rations were formulated to be around 18% for Hp and about 15% for Lp. The indigestible NDF, as % of the NDF, of the two oat hays used were 40.8% for Ld hay and 35.54% for the Hd hay. Wheat straw was included in the Ld diets to obtain iso-NDF diets of different quality. Cows were fed *ad libitum* for 14 days with data collection taking place over the last 4 days of each period.

The aim of this study was to improve nitrogen use efficiency (NUE) and to investigate the possible economic benefits for dairy farmers presented through better nutritional management by optimizing the use of dietary protein by using better quality forages (with regard to the digestible NDF) and by reducing CP intake by formulating the diets to meet metabolisable protein (MP) requirements.

Dry matter intake (DMI) and milk yields (MY) were recorded daily and DMI for 3 of the diets was found to be similar, with the exception of the LpLd diet (i.e. LpLd had lower DMI than the other 3 diets) showing how protein availability can counteract the lower forage quality, by stimulating fibrolytic bacteria. Energy corrected milk yield (ECM) was found to drop 2.46kg/d for Hd diets and 3.00kg/d for Ld diets with Hp having higher production then Lp levels. Nitrogen use efficiency was found to improve by 3.04% when protein was reduced in combination with Hd forages and by 5.63% for Ld forages. Dry matter intakes and milk yields were used to determine daily feed costs and income respectively. These were used to calculate income over feed cost (IOFC). It was seen the higher protein diets had a higher cost per day but also resulted in higher milk production. The impact of better quality forages can also clearly be seen, especially on the lower protein levels. Statistically diet had no effect on IOFC, with IOFC being the same across all treatments.

We concluded that lowering protein improved NUE significantly with forage digestibility contributing to the level of improvement. However, a consequence of reducing CP was a corresponding decrease in production. Although no statistical difference was found for IOFC,

numerical differences that would be considered significant on farm level were however observed. The lack of statistical significance is a possible consequence of high standard errors of the mean (SEM) resulting from limited data point and thus we recommend performing this study on a larger herd to improve statistical variation.

Uitreksel

Besoedeling vanaf melkboerderye, in die vorm van stikstof uitskeiding word 'n toenemende kommer wêreldwyd as gevolg van kweekhuisgasse en aardverwarming. Baie lande wêreldwyd, veral in die Europese Unie (EU), het reeds wetgewing in plek gestel met betrekking tot die verwerking van melkkery storting in 'n poging om stikstof besoedeling te verminder. Vele studies is reeds onderneem om moontlike verlaging van stikstofbesoedeling te weeg te bring. In Suid-Afrika is daar tans geen regulasie ten opsigte van die storting van afvalstowwe nie. Dit sal egter na alle warskynlikheid die EU voorbeeld binnekort volg.

Vier lakterende Holstein koeie is gebruik in 'n 4 x 4 Latynse vierkant ontwerp gebalanseer vir oordrag effekte met 'n 2 x 2 faktoriale behandeling. Behandelings was lae ruproteïen (RP) konsentrasie met 'n hoë neutraal bestande vesel (NBV) verteerbaarheid (LpHd), 'n hoë RP konsentrasie met 'n hoë NBV verteerbaarheid (HpHd), lae RP konsentrasie met 'n lae NBV verteerbaarheid (LpLd) en 'n hoë RP konsentrasie met 'n lae NBV verteerbaarheid (HpLd). Ruproteïen konsentrasies vir die rantsoene is geformuleer om ongeveer 18% te wees vir Hp en 15% vir Lp. Die onverteerbare NBV, as 'n % van NBV, van die twee hawerhooi bronne wat gebruik was, is 40.8% vir Ld hooi en 35.54% vir die Hd hooi. Koringstrooi is ingesluit in die Ld dieet om gelyke-NBV diëte te kry met verskillende verteerbarhede. Koeie is ad libitum gevoer vir 14 dae op elke dieet met data-kolleksie oor die laaste 4 dae van elke tydperk.

Die doel van hierdie studie was om stikstofgebruiks-doeltreffendheid (SGD) te verbeter en om die moontlike ekonomiese voordele vir melkboere te ondersoek: eerstens, deur beter voedingswaardebestuur en die optimalisering van die gebruik van proteïen met behulp van beter verteerbare ruvoer (NBV) en tweedens, deur die vermindering van RP inname deur die diëte te formuler om metaboliseerbare proteïen (MP) vereistes te voldoen.

Droëmateriaal inname (DMI) en melkproduksie (MP) is daagliks aangeteken en DMI vir 3 van die diëte was soortgelyk aan mekaar met die uitsondering van die LpLd dieet. Die LpLd het 'n laer DMI as die ander 3 diëte gehad, wat daarop dui dat hoeproteïen beskikbaarheid die laer kwaliteit ruvoer teenwerk deur die stimulering van veselverteerende bakterieë. Energiegekorrigeerde melkproduksie (EGM) was 2.46kg / d laer vir Hd diëte en 3.00kg / d vir Ld diëte resepktiewelik met hoër melk produksie vir Hp diete teenoor Lp diëte. Die vermindering van proteïen in kombinasie met Hd en Ld ruvoer het stikstofgebruik-doeltreffendheid met 3.04% en met 5.63% onderskeidelik vermeerder. Droëmateriaal inname en melkproduksie is gebruik om daaglikse voerkoste en -inkomste te bepaal en om marge bo voerkoste (MBVK) te bereken. Hoër proteïen diëte het beide 'n hoër koste per dag gehad en 'n hoër melkproduksie gehad. Die invloed van hoër kwaliteit ruvoer is duidelik, veral met die laer proteïen diëte. Geen statistiese verskille is met MBVK tussen behandelings waargeneem nie

Ons gevolgtrekking was dat die verlaging van proteïen vlakke die SGD verbeter het en dat ruvoerverteerbaarheid 'n beduidende bydrae gemaak het. Verlaagte RP het egter melkproduksie onderdruk. Hoewel geen statistiese verskil vir MBVK gevind is nie, was daar wel numeriese verskille wat op plaasvlak 'n beduidende verskil sal maak. Weens 'n gebrek aan 'n groot genoeg datastel en die gevolglike hoë standard afwyking, is geen statistiese betekenisvolheid tussen behandelings waargeneem nie.

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Notes

The language and style used in this thesis is in accordance with the requirements of the *Journal of Dairy Science.* This thesis represents compilation of manuscripts in which each chapter is treated as an individual entity and therefore some repetition between chapters is unavoidable.

List of Abbreviations

AA	Amino acids
ADF	Acid detergent fibre
ADL	Acid detergent lignin
CF	Crude fibre
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
dNDF	Digestible neutral detergent fibre
EAA	Essential amino acids
ECM	Energy corrected milk yield
ECP	Endogenous crude protein
Eeff	Energy efficiency
iNDF	Indigestible neutral detergent fibre
IOFC	Income over feed cost
MCP	Microbial protein
MNE	Milk nitrogen efficiency
MP	Metabolisable protein
N	Nitrogen
NDF	Neutral detergent fibre
Neff	Nitrogen efficiency
NFF	Non-forage fibre

NH$_3$	Ammonia
NPN	Non-protein nitrogen
NUE	Nitrogen use efficiency
OM	Organic matter
peNDF	Physical effective neutral detergent fibre
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
TP	True protein
uN	Unavailable nitrogen
VFA	Volatile fatty acids

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Chapter 1:
General Introduction

The scope of dairy farming in South Africa has been changing in recent years. It is becoming more difficult for smaller scale farmers to remain competitive in the dairy industry due to increasing feed and processing costs combined with relatively small increases in milk price. As a result, smaller scale farms are being sold off to larger commercial farms that can do much of their own processing on farm and dilute fixed costs. Thus, there are now fewer dairy farms but much larger dairy herds per farm (Milk South Africa, 2014). Due to the increasing demand for milk and milk products, effort is being placed on increasing animal production. This increased production raises the need for nutrient rich diets that can meet the needs, particularly for energy and protein, of these high producing cows. As a result, crude protein (CP) levels in dairy rations are often as high as 18 to 20% as data published in NRC (2001). The NRC associates this increase in CP with a corresponding increase in milk production up to roughly 20% CP. This however results in a significant increase, up to 75%, of the dietary N being excreted in the urine and faeces (Arriola Apelo *et al.*, 2014).

Locally as well as globally, these intensive dairy production systems produce large amounts of pollutants of which the main concerns arise from nitrogen (N), phosphorus (P) and methane (CH_4) (Kohn *et al.*, 1997). Cattle farming, especially dairy farming, is considered to be the largest animal husbandry source of ammonia (NH_3) (Bussink and Oenema, 1998). Airborne N emissions can result in a negative impact on air quality and may also damage habitats, resulting in loss of species diversity. Along with this, manure runoff and leaking from manure pools known as lagoons, which are common on commercial dairy farms, can pollute nearby water sources. With increasing intensification in dairy farming systems, nitrogen use efficiency (NUE) of dairy cows has gained increasing attention in recent years largely due to the environmental concerns over N emissions which are among the largest contributors to atmospheric pollution in the form of greenhouse gasses such as nitrous oxide (N_2O) (Kebreab *et al.*, 2001).

Rumen microbes are able to break down proteins and non-protein nitrogen (NPN) to utilise the inorganic N from the NH_3 which they produce. This would otherwise be a toxic waste product which the animal would have to excrete, however, the microbes utilise it to synthesise amino acids (AA) and proteins needed for microbial growth (Patton *et al.*, 2014) and in turn produce usable protein, in the form of microbial protein, for the ruminant. The more efficiently this process is carried out, the lower the levels of N waste become that are excreted into the environment. Sufficient energy is needed for the microbes to efficiently utilise the N in the rumen (Oldham, 1984). The rumen microbes are able to digest large amounts of plant based material, i.e. fibre, which is indigestible to normal mammalian enzymes. They then convert it into usable energy through fermentation

processes (Dewhurst *et al.*, 2000). Fibre in the diet also plays an important role in feed flow rate through the rumen which also affects the microbe's effectiveness at utilising the N available in the rumen.

Nitrogen use efficiency is an expression of the ratio between N used to synthesise milk protein and the dietary N intake (Higgs *et al.*, 2013). This efficiency of N use for dairy production systems rarely exceeds 25 to 30%, i.e. approximately 70% of ingested N is excreted into the environment (Lapierre and Lobley, 2001; Ipharraguerre *et al.*, 2005) with approximately 25% of the N being converted into milk (Arriola Apelo *et al.*, 2014). One way to achieve better NUE is to reduce dietary CP intake (Nielsen *et al.*, 2003; Kalscheur *et al.*, 2006) which subsequently reduces the excreted N levels (Børsting *et al.*, 2003). However, reducing CP intake on high producing dairy cows can have negative effects on dry matter intake (DMI) and this in turn will reduce milk production (Fisher, 2002; Hristov *et al.*, 2005). Therefore, care must be taken in balancing diets where protein concentrations are lower to prevent a decrease in DMI.

The efficiency with which protein is utilised in the rumen is largely dependent on the type of carbohydrate being used as an energy source. While the overall amount of N excreted by a dairy cow is directly related to the amount of N the cow consumes in its diet, the pathway through which it is excreted (urinary or faecal) is rather determined by the type of carbohydrate and type of forage (Weiss *et al.*, 2009). Also, increasing MP increases N excretion in both faeces and urine, but the increase is larger in urine. It has been suggested that MP should be balanced carefully as not to exceed the cow's requirements which would increase N excretion but at the same time not underestimate MP as this could negatively affect milk production.

 In conjunction to the benefits that improved NUE presents to environmental conditions, it also holds economic benefits for the farmer. Shalloo *et al.* (2004) showed that feed cost contributes to more than 50% of total dairy production costs with protein being one of the most expensive feed components. With current feed prices still on the rise, particularly those used in concentrate feeds, interest in substituting energy-rich grains with high-quality forages and optimizing the use of dietary protein sources is growing rapidly (Hymøller *et al.*, 2014). In turn, the optimization of protein use will assist in reducing the environmental impact of dairy farming by improving NUE (Børsting *et al.*, 2003; Yan *et al.*, 2010).

A study was carried out at Welgevallen, Stellenbosch University's experimental farm, to determine the effect of forage fibre quality on the efficiency with which dairy cows are able to utilise protein in their diets. The aim of this study was to address, firstly and most importantly, the environmental factors involved in dairy feed management through the principle of improved NUE and secondly the possible economic benefits that improved NUE may present to the dairy industry. It aimed to achieve this outcome through combining two different methods. Firstly, optimizing the use of dietary protein using forages of different quality with regard to the digestibility of neutral detergent fibre (NDF) and then determining the effects that these various qualities have on NUE. In

order to achieve this, two protein levels were tested in combination with forages that displayed varying levels of digestible NDF (dNDF). Secondly, reducing CP intake by formulating the diets to meet metabolisable protein (MP) requirements to bring about a decrease in N excretion with minimal effects of milk yield and quality.

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Chapter 2:
Literature Review

2.1. Introduction

Dairy production systems produce large amounts of pollutants of which the main concerns arise from nitrogen (N), phosphorus (P) and methane (CH_4) (Kohn *et al.*, 1997). Airborne N emissions can result in a negative impact on air quality and also damage habitats, resulting in loss of species diversity. Along with this, manure runoff from dairy farms in particular can pollute nearby water sources. With increasing intensification in dairy farming systems, nitrogen use efficiency (NUE) of dairy cows has gained increasing attention in recent years largely due to the environmental concerns over N emissions which are among the largest contributors to atmospheric pollution in the form of greenhouse gasses such as nitrous oxide (N_2O) (Kebreab *et al.*, 2001).

Due to the microbial activity of the rumen, large amounts of plant based material which are indigestible to normal mammalian enzymes, can be converted to usable energy through fermentation processes (Dewhurst *et al.,* 2000). This additional energy enables microbes to better utilise the inorganic N from ammonia (NH_3) (Oldham, 1984), which would otherwise be a toxic waste product, to synthesise amino acids (AA) and proteins needed for microbial growth (Patton *et al.,* 2014) and in turn produce usable protein for the ruminant animal from these toxic N waste products. The more efficiently this process is carried out, the lower the levels of N waste become that are excreted into the environment, hence the attention to NUE.

Nitrogen use efficiency is an expression of the ratio between N used to synthesise milk protein and the dietary N intake (Higgs *et al.*, 2013). This efficiency of N use for dairy production systems rarely exceeds 25 to 30%, i.e. approximately 70% of ingested N is excreted into the environment (Lapierre and Lobley, 2001; Ipharraguerre *et al.*, 2005). One way to achieve better NUE is to reduce dietary crude protein (CP) intake (Nielsen *et al.*, 2003; Kalscheur *et a*l., 2006) which subsequently reduces the N levels in excrement (Børsting *et al.*, 2003). However, reducing CP intake on high producing dairy cows can have negative effects on dry matter intake (DMI) and this in turn will reduce milk production (Fisher, 2002; Hristov *et al.*, 2005).

In conjunction with the benefits that improved NUE presents to environmental conditions, it also holds economic benefits for the farmer. In Shalloo *et al.* (2004) it was demonstrated that feed cost contributes more than 50% of total dairy production costs. With current feed prices still on the rise, particularly those used in concentrate feeds, interest in substituting energy-rich grains with high-quality forages and optimising the use of dietary protein sources is growing rapidly (Hymøller *et al.*, 2014). In turn, the optimisation of protein use will assist in reducing the environmental impact of dairy farming by improving NUE (Børsting *et al.*, 2003; Yan *et al.*, 2010).

2.2. Protein nutrition in dairy cows

2.2.1. Introduction

Crude protein in feedstuffs is defined as the N content × 6.25, assuming on average that feedstuffs contain 16g of N per 100g of protein (NRC, 2001). This means, however, that the CP values include both protein N and non-protein N (NPN). There are two basic fractions for dietary CP, the first fraction consists of true protein (TP) and NPN that can be degraded in the rumen for microbial use. This is known as rumen degradable protein (RDP). The second fraction consists of proteins that pass out of the rumen and can be digested post-ruminally providing the component amino acids (AA) that are absorbed by the small intestine or need to be broken down and detoxified for excretion. This is known as rumen un-degradable protein (RUP). A third fraction that has come to light more recently is one known as unavailable nitrogen (uN) (Ross *et al.*, 2013). This fraction consists of N (or CP) that cannot be digested at all in the gastrointestinal tract (GIT). The significance of this fraction is that some protein sources considered to be high in RUP may also be high in uN, thus resulting in a much lower true usable protein value than previously expected. The true usable protein that passes into the small intestine and is available to supply the AA building blocks needed for maintenance, growth, reproduction and lactation is known as metabolisable protein (MP) and consists of three components namely microbial crude protein (MCP), RUP and, to a much smaller extent, endogenous crude protein (ECP) (NRC, 2001; Patton *et al.*, 2014).

2.2.2. Protein metabolism in the dairy cow

Studies relating to N usage in dairy cattle have been investigated quite extensively in various reviews and reports (Oldham, 1984; Lapierre *et al.*, 2005). Figure 2-1 details the protein metabolism and N pathways within a dairy cow. When feeding dairy cows, the first system being fed is the microbial system in the rumen. Dietary proteins and NPN, along with endogenous proteins and lysed microorganisms in the rumen make up the pool of CP that can be potentially fermented. It has been demonstrated that all protein degradation in the rumen is due to microbial enzymatic activity through the numerous strains of bacteria, protozoa and anaerobic fungi which release various proteases, peptidases and deaminases (Wallace, 1996). The peptides and AA released due to proteolytic enzyme digestion, if not used directly in microbial protein synthesis, are fermented to NH_3 and carbon skeletons which, along with N derived from NPN, provide the N building blocks needed for growth by the ruminal microorganisms (Patton *et al.*, 2014). The cellulolytic bacteria require mostly NH_3 as their N source for growth whereas the amylolytic bacteria make greater use of peptides and AA. Protozoa, on the other hand, are predatory and engulf and digest rumen bacteria. High producing dairy cows are at times fed large quantities of good quality protein but because the rate of protein degradation is not coupled to that of AA, NPN and NH_3 assimilation into MCP, catabolism of AA and peptides results in excess NH_3 in the rumen

that is absorbed through the rumen wall and ultimately either recycled back, via urea, or lost by urinary N excretion (NRC, 2001; Sannes *et al.*, 2002). The microbial production efficiency can be defined as the amount of MCP in grams (g) passing into the intestine per kilogram (kg) of degraded organic matter (OM) (Oba and Allen, 2003).
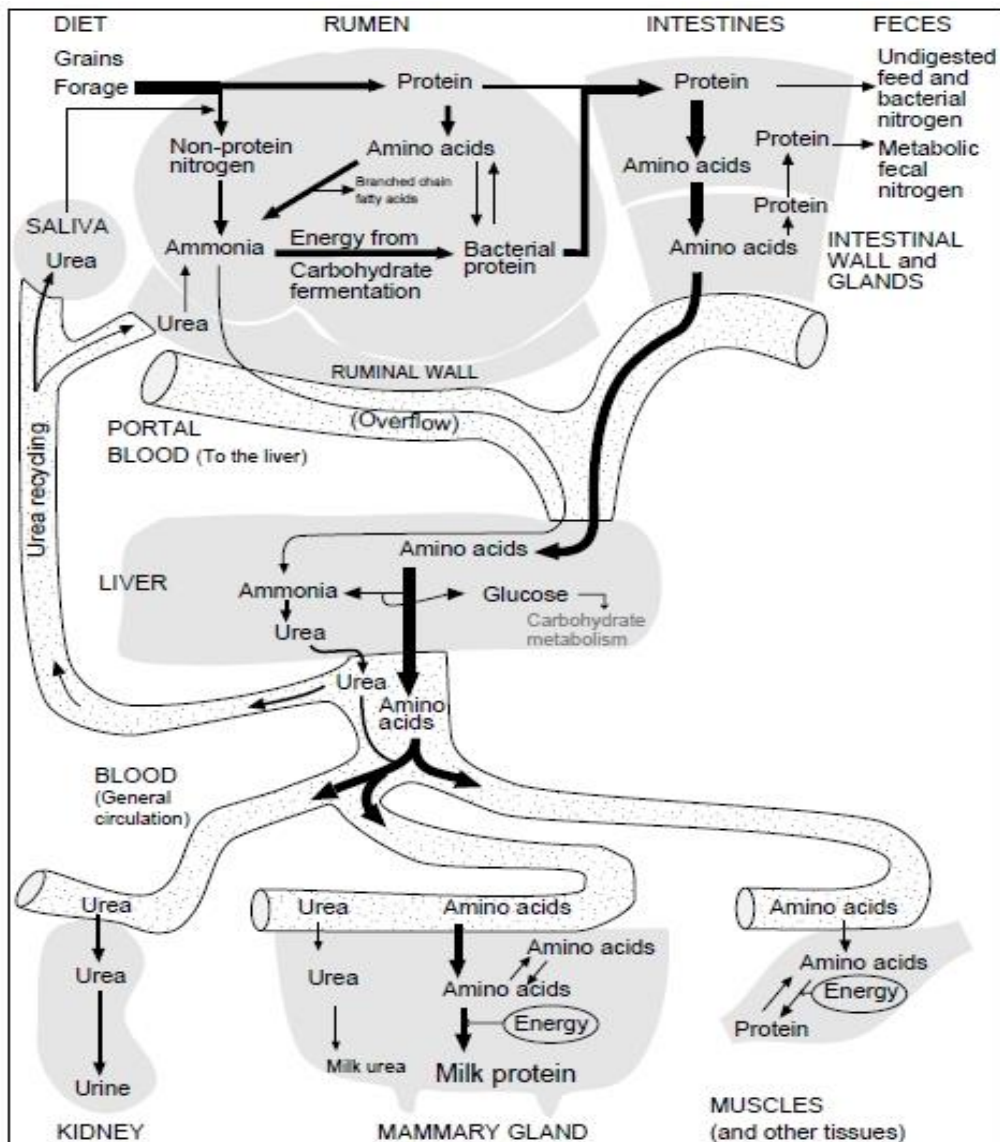
Figure 2-1. Protein metabolism in dairy cows. Adapted from Babcock Institute, Department of Dairy Science; University of Wisconsin-Madison.
https://federated.kb.wisc.edu/images/group226/52745/5.ProteinMetabolisminDairyCows.pdf

The second system that must be fed in a lactating cow is the mammalian system which requires AA but also needs to detoxify any excess NH_3 that managed to pass out of the rumen. The fraction of protein that ends up in the intestine and is available for use by the cow is known as the MP. This consists of dietary protein that is not degraded by the rumen microbes, MCP and also small amounts of the peptides and AA that have not been incorporated into microbial production

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and escape the rumen before they are degraded into NH_3 (NRC, 2001; Patton *et al.*, 2014). The abomasum and small intestine of ruminants' function in the same way as the digestive system of monogastric animals where strong acid and various digestive enzymes are secreted and break down RUP and MCP into AA which can be absorbed through the small intestine and used by the animal for protein synthesis.

2.2.3. Protein requirements in lactating dairy cows

Lactating dairy cows have a high protein requirement due to their need of AA for milk protein synthesis. Diets with protein levels below 14% CP begin to limit microbial activity in the rumen and thus negatively affect digestion which will in turn limit DMI (Fisher, 2002; Hristov *et al.*, 2005; Alstrup *et al.*, 2014). This drop in DMI has been found to increase with corresponding decreases in CP levels (Weiss *et al.*, 2009). Even though the rumen and ruminant animal can function with NPN as the only source of N (Virtanen, 1966), additional supply of essential AA (EAA) is necessary if the high milk production of current day dairy herds is to be maintained. This EAA composition is of significant importance to dairy cows receiving diets supplying MP that is close to or just below NRC requirements (Giallongo *et al.*, 2015). The NRC (1994, 1998) showed that optimum AA profiles exist for poultry and swine in MP for the various physiological stages of these animals. These profiles are assumed to be true for dairy cows too. It is however much more difficult to define EAA requirements for dairy cows due to the alterations that nutrients undergo in the rumen by the microorganisms (Lapierre *et al.*, 2006). In a collection of studies reviewed by Lee *et al.* (2015) it was noted that Methionine, Lysine and Histidine are three of the most limiting AA for dairy cows in a variety of intensive production systems. Despite this, protein requirements for dairy cows are still expressed as MP rather than EAA. So, when animals are fed to meet MP requirement, they are likely over fed many EAA to ensure meeting the requirement of other key EAA which results in poor NUE (Arriola Apelo *et al.*, 2014). MCP is the major factor affecting both quantity and quality of MP being absorbed from the small intestines. This is because RUP can have a lower digestibility in the small intestine than MCP (Oba and Allen, 2003) which is highly digestible and has an AA profile closely matching that of the dairy cow's requirements (O'Connor *et al.*, 1993). However, it should be noted that the traditional invasive *in vivo* methods for determining the parameters needed to estimate MCP flow to the intestine do exist but are not only complicated and expensive, but were also found to have unknown accuracy (Titgemeyer, 1997; Dewhurst *et al.*, 2000). Of the N consumed by a dairy cow, approximately 25 to 30% is used for milk protein and the majority of the remaining N is excreted (Hristov *et al.*, 2004; Ipharraguerre *et al.*, 2005). Data from the NRC (2001) shows that an increasing CP level in the diet will result in increased milk production until a maximal milk yield is reached at approximately 23% CP after which further increases will cause production to decline (Figure 2-2). However, the levels of excess N excreted increase nearly linearly in correspondence to the increase in dietary CP (Figure 2-3).
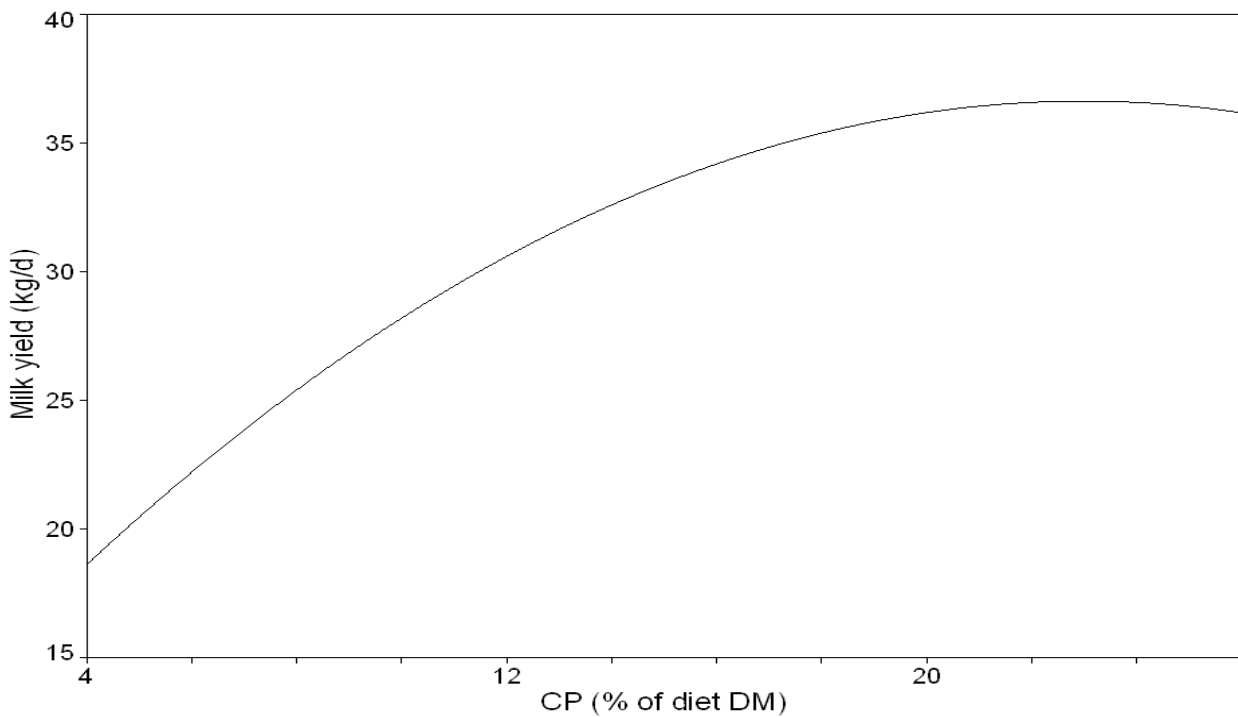
Figure 2-2. Milk yield (kg/d) versus CP (% of diet DM) using the equation: Milk yield (kg/d) = (0.8*DMI+2.3*CP-0.05*CP2-9.8) from NRC (2001), with DMI at 25 kg/d.
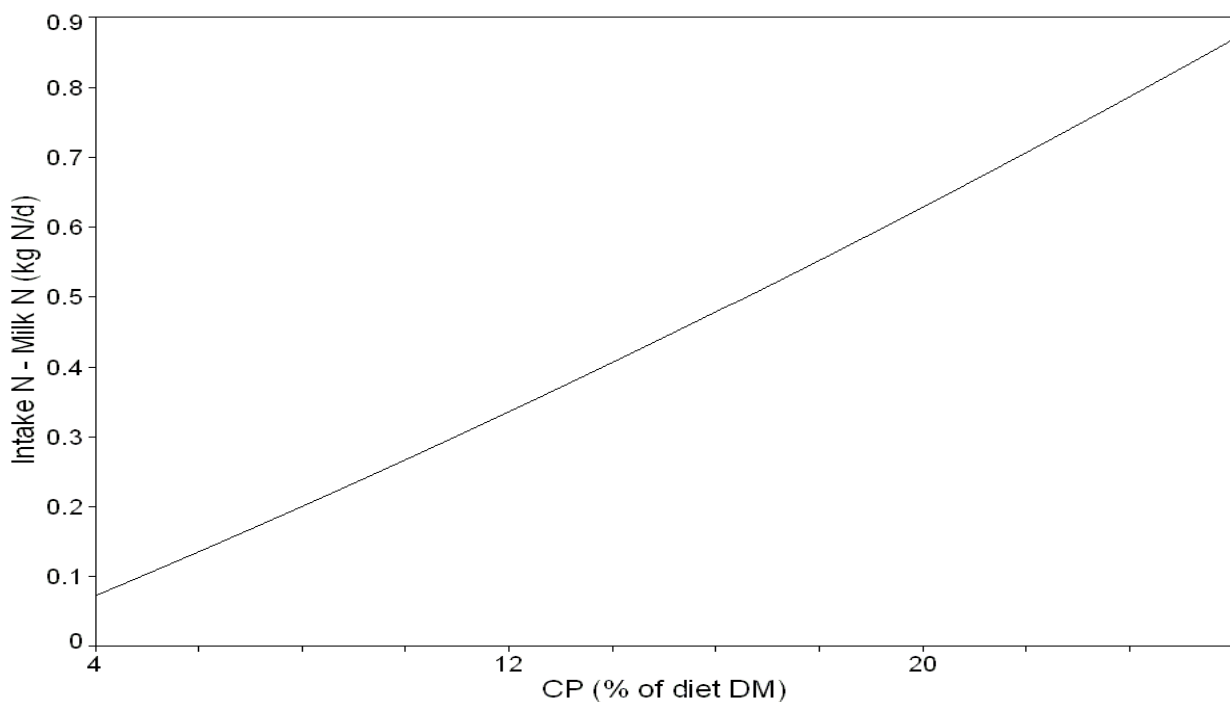
Figure 2-3. Nitrogen not used for milk production (Intake N – Milk N) in kg N/d versus CP (% of diet DM) using the equation: Milk yield (kg/d) = (0.8*DMI+2.3*CP-0.05*CP2-9.8) from NRC (2001) for milk yield and assuming 2.95% milk protein and 25 kg DMI/d.

The aim of protein nutrition in dairy cows is to provide sufficient RDP, balancing feed proteins with NPN supplements, to meet but not exceed the needs of the microorganisms in the rumen to achieve optimal rumen functionality with maximal MCP synthesis and also to provide the correct types and amounts of RUP in order to optimise the profiles and levels of AA absorbed in the small intestines (NRC, 2001; Lee *et al.*, 2015). The NRC (2001) lists the CP requirements of large breed cows (~650kg) in mid lactation (approximately 90 days in milk) as between 14.1 and 18.5% in relation to milk production levels from 35 to 55 kg/day with DMI between 22.7 and 31.7kg/day. However, the efficiency with which this CP is utilised in the rumen is largely dependent on energy (Oldham, 1984).

2.2.4. Nitrogen excretion

Approximately 70% of ingested N remains unused by the cow and needs to be excreted as waste (Lapierre and Lobley, 2001; Ipharraguerre *et al.*, 2005). Excess N exists in various forms and this is what determines its excretory pathway. In the rumen, NH_3 is produced from deamination of AA and peptides and from dietary NPN sources. Most of the excess NH_3 is absorbed through the rumen wall into the blood stream. Ammonia is however toxic to the animal so it needs to be transported to the liver where it is converted to urea (Lapierre *et al.*, 2005). The process involved in converting excess protein into urea is very energy demanding (Alstrup *et al.*, 2014). Urea is then excreted through the kidneys in the form of urine.

From the rumen, the various forms of N remaining pass through to the intestine where AA are absorbed to synthesise proteins for various metabolic processes. The remaining undigested feed and microbial N, along with metabolic faecal N is excreted in the faeces as well as small amounts of NH_3 that are excreted as gas. In a review by Lapierre and Lobley (2001), it was shown that approximately 35% of the excess N was excreted via faeces and 34% via urine.

2.2.5. Nitrogen recycling

As discussed above, NH_3 is converted to urea in the liver and returned to the blood stream where it is mostly excreted through the kidneys. Hepatic synthesis of urea can exceed the apparent digestible N which would results in a negative N balance if no mechanism existed to salvage some of the losses (Lapierre and Lobley, 2001). In dairy cows, as much as 73% (Recktenwald *et al.*, 2014) of this urea can once again enter the GIT where it can be converted back to NH_3 and used for the synthesis of microbial protein (Harmeyer and Martens, 1980; Lapierre and Lobley, 2001; Stewart and Smith, 2005; Røjen *et al.*, 2008). In a study by Hvelplund and Beck (1999), N and carbohydrate intake were synchronised for low producing dairy cows. The cows were fed 12% and 16% CP concentrate supplements. It was found that NH_3 concentration,

cellulolytic activity and concentration of short chain fatty acids remained unaffected between the two diets. It was thus concluded that N-recycling was able to supply sufficient N to rumen microorganism for lower producing animals. An approach to improve NUE is to reduce urea-N excretion in urine by stimulating urea-N re-entry into the rumen for use in microbial protein synthesis (Recktenwald *et al.*, 2014). It has been observed that dietary carbohydrate can stimulate urea-N re-entry into the rumen. This is most likely due to rumen N depletion resulting from microbial growth gained from the carbohydrate energy (Al-Dehneh *et al.*, 1997). It has also been suggested that carbohydrate fermentation may have a secondary effect by altering the permeability of the rumen wall through production of volatile fatty acids (VFA's) and CO_2 (Abdoun *et al.*, 2006) as both of these by-products have been shown to stimulate urea-N re-entry and may play a role in controlling N supply for microbes when fermentable carbohydrate concentration in the rumen is high (Remond *et al.*, 1993). Another pathway through which N is recycled is via saliva production. A portion of the hepatic urea output, 22% as shown by Maltby *et al.* (2005), is transported from the blood to the saliva where it is ingested again as the cow eats or ruminates.

Figure 2-4 presents the pathways of various forms of N in the dairy cow. The two N-recycling pathways by which N can enter the GIT are as blood urea being absorbed through the GIT walls or as endogenous secretions of saliva (Lapierre and Lobley, 2001).
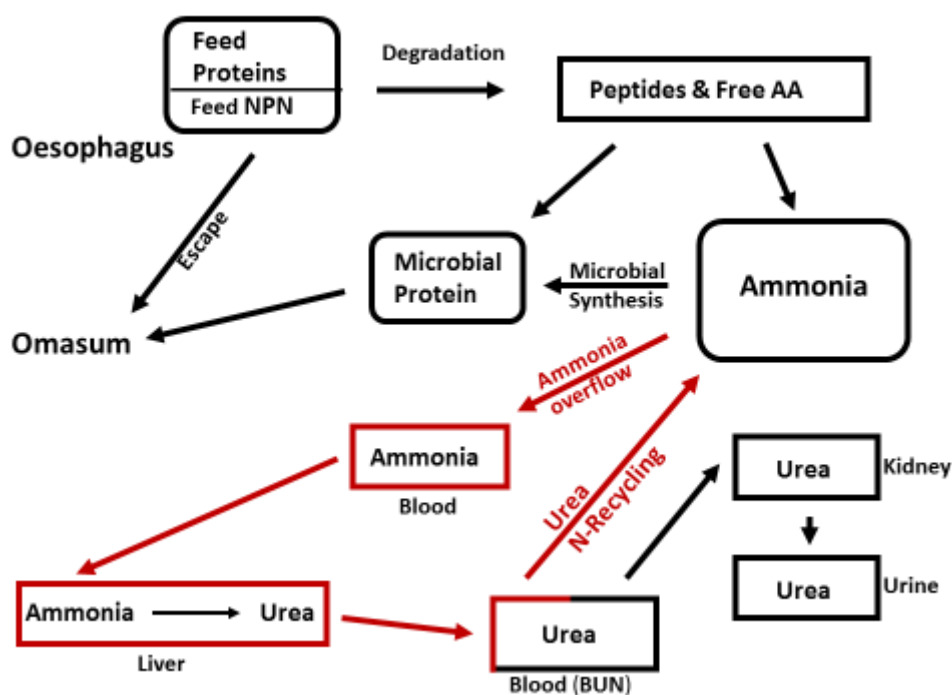
Figure 2-4. Nitrogen pathways in the ruminant. Adapted from (Lapierre; Lobley, 2001)

2.3. Fibre digestion in dairy cows

2.3.1. Introduction

Carbohydrates are the largest component of a dairy cow's diet and, from a dairy nutrition perspective, they are classified into two subdivisions namely non-structural carbohydrates (NSC) or structural carbohydrates. The latter is most accurately represented by Neutral Detergent Fibre (NDF). A dairy diet usually contains 35% NSC which serves as the main energy source for high producing cows. The NDF fraction should be an absolute minimum of 25% but 30 to 35% is recommended (NRC, 2001).

The proximate analysis value for crude fibre (CF) and nitrogen free extract (NFE) are not very accurate when being applied to forage as they do not accurately separate the carbohydrate fractions into the true fibrous component and the non-fibrous component (Soest, 1994). The method for determining acid detergent fibre (ADF) quickly replaced CF as it was easier and gave similar results. However, ADF isolated cellulose and lignin but not hemicellulose and was thus not suitable for determining total structural fibre. Due to inaccuracies associated with CF determination and the similar components that ADF consists of, these values are not considered nutritionally very meaningful for ruminants (NRC, 2001; Udén *et al.*, 2005).

A more useful system was developed in the 1960 and 1970's's by Dr Peter van Soest and was refined over a number of years (Mertens, 2002; Udén *et al.*, 2005). With this method, he separated the cell contents from the cell wall constituents by refluxing the forage in a neutral detergent which would solubilise the digestible cell contents leaving what is now known as NDF. This fraction consists of cellulose, hemicellulose, lignin and low amounts of silica and cutin. It can further be refluxed in an acid detergent which dissolves hemicellulose leaving the ADF fraction. This fraction can be further broken down by a 72% sulphuric acid solution which dissolves the cellulose leaving the fraction known as acid detergent lignin (ADL). Thus, he devised methods that can completely classify all the cell wall contents (Mertens, 2002; Udén *et al.*, 2005). Table 2-1 details the difference between constituents of the proximate analysis and the detergent system fractions.

Table 2-1. Carbohydrate fractions**.**

Proximate Component	Chemical fraction		Detergent fraction
Nitrogen-free Extract	Sugar		**Cell Contents** **(NFC)**
	Starch		
	Pectin		
	Hemicellulose		
Crude Fibre	OH Soluble	Lignin	**ADF** **Cell wall** **(NDF)**
	OH Insoluble		
	Cellulose		
Ash	Detergent Insoluble Ash		

2.3.2. Carbohydrate metabolism in dairy cows

The quantity and quality of forage based NDF in the diet and its effect on NUE in dairy cows has not been very well documented in literature but rumen fermentation of NDF and the various metabolites produced are well known. Figure 2-1 below details carbohydrate metabolism and its various fermented and digested end products throughout the ruminant.

Non-structural carbohydrates include sugars, starches, pectins, β-glucans and fructans. The Cornell Net Carbohydrate and Protein System (CNCPS) defines NSC as [100 - ((%NDF + %CP + %Fat + Ash)]. These soluble carbohydrates are rapidly broken down by amylolytic bacteria in the rumen and provide a significant energy source for microbial production. Fibre is digested by cellulolytic bacteria and is digested more slowly than NSC. Microbial fermentation of carbohydrate by these various bacteria produce 3 major VFA's, namely acetate, propionate and butyrate, which are constantly absorbed through the rumen wall and are transported via ruminal veins to the portal vein passing through the liver. The three VFA's have distinct metabolic fates. Propionate is almost completely used in the liver for gluconeogenesis which is critically important as virtually no glucose passes through the rumen, however excessive propionate can cause negative effects on performance, mainly from reduced DMI (Oba and Allen, 2003). Acetate is an important source of Acetyl CoA which is necessary for the synthesis of lipids. However, it is also oxidised throughout the body of the cow to generate ATP. Butyrate, which is mostly in the form of β-hydroxybutyric acid, is also oxidised by various body tissues for energy production.
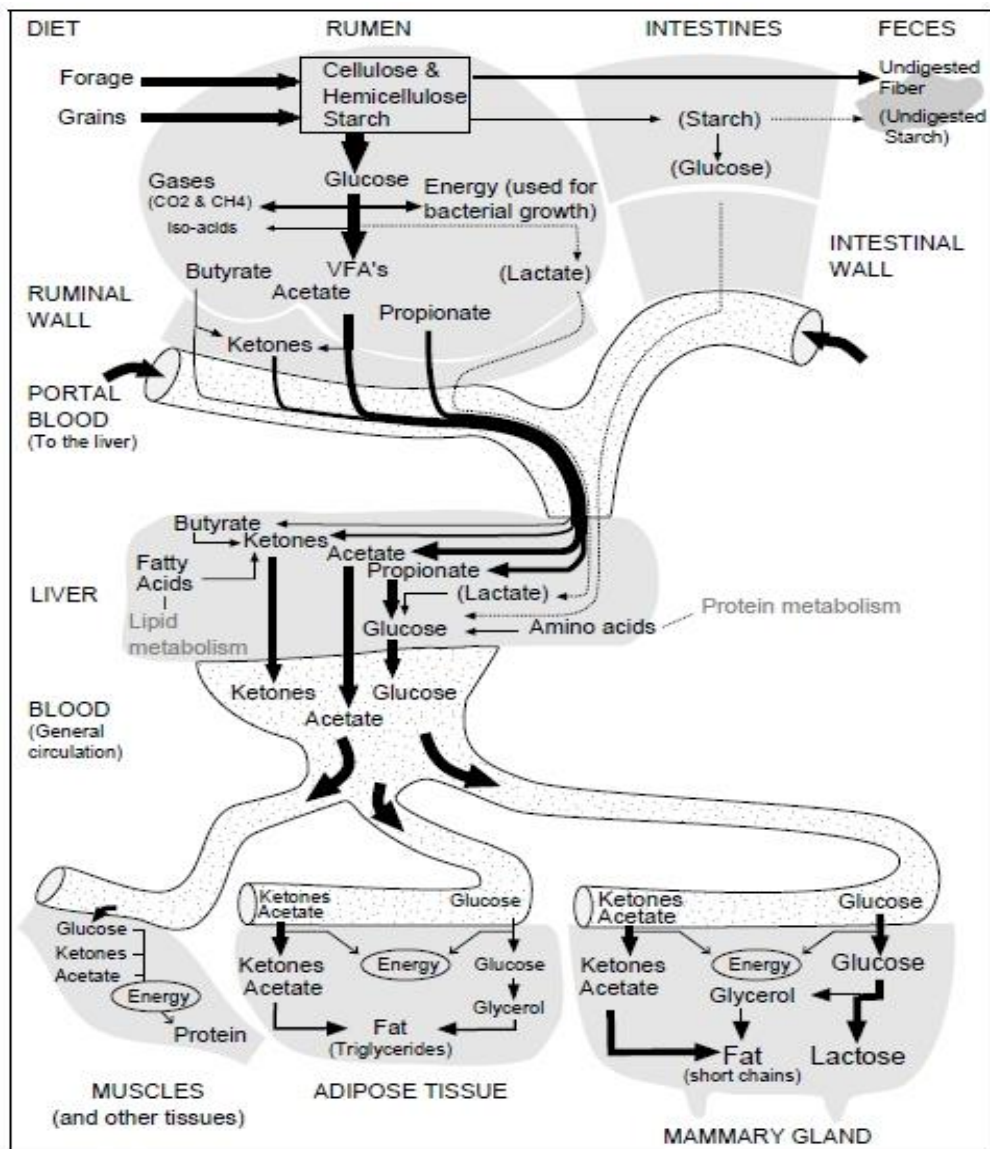
Figure 2-5. Carbohydrate metabolism in the ruminant. Adapted from Babcock Institute, Department of Dairy Science, University of Wisconsin-Madison. https://federated.kb.wisc.edu/images/group226/52745/3.CarbohydrateMetabolisminDairyCows. pdf

Fibrolytic bacteria are a lot more sensitive to acidic conditions than amylolytic bacteria, therefore if the content of NSC in the diet is too high, the rumen pH will drop due to the rapid production of VFA as well as lactic acid which is produced by Streptococcus bovis which thrives in the presence of large amounts of starch and acidic conditions. This drop in pH will have a significantly negative impact on fibrolytic bacteria attaching to fibre and the rumen microbial population will change from fibrolytic to predominantly amylolytic and can result in ruminal acidosis (Sung *et al.*, 2007). Thus, fibre digestion, and consequently the production of acetate which is associated with it, will decrease. Some starch however passes through the rumen into the intestines. This is either rumen undegradable starch or small amounts of starch that passed

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through the rumen before the microbes could fully ferment it. Starch in the intestine is digested by the secretion of pancreatic amylase, similarly to monogastric animals.

2.3.3. Fibre requirements in dairy cows

Lactating cows have a minimum fibre requirement to maintain optimum gut health and production. However, ruminal digestion of forage can be less than 25% to over 75% depending on the forage type and also within a particular forage type as it is influenced by environment and age of the forage at harvest and its particle size when fed. The main ingredient for energy also affects the required level of NDF (Oba and Allen, 1999; NRC, 2001). This results in various requirements for forages depending on the feed, however the NRC indicates that dairy cows require 30 to 35% NDF with a minimum of 22% the of dietary NDF coming from forage fibre (NDF) sources. In general, forages make up to 50% or more of the diet and have a large influence on energy and carbohydrate intake. Management of digestible carbohydrate intake from forage is extremely important as energy requirement for maintenance and milk production for high producing cows often exceeds the amount of energy that these cows can consume (Kendall *et al.*, 2009). The reticulorumen rumen is known to have stretch and touch receptors in its wall, thus when feeds with lower digestion rates accumulate in the rumen, these receptors result in a negative impact on DMI (Allen, 1996). Neutral detergent fibre is thus considered to be the primary dietary impact on the physical fill effect due to its generally lower rates of digestion (NRC, 2001). The digestibility of NDF is an important parameter of forage quality because physical fill plays a major part in limiting DMI, which is significant because the fibrous component of forage remains in the rumen much longer than the non-fibrous component. Thus, NDF digestibility influences animal performance independent of the NDF concentration of the forage. In studies on brown midrib mutant corm silage, it was shown that silage with higher NDF digestibility resulted in higher DMI as a result of reducing the time of physical fill in the rumen and allowing for increased voluntary feed intake which in turn relates to increased milk yield (Oba and Allen, 2000b). In addition to DMI, increased digestibility can increase the energy density of the diet allowing for better NUE through increased microbial production in the rumen. When feeds are digested, fermentation acids are released in the rumen which lower pH. Therefore, when desired increase in fermentation is achieved, these acids need to be removed or neutralised. Cows secrete more salivary buffer while chewing therefore buffering capacity of rumen digesta is dependent on the total chewing time (Oba and Allen, 2000a). Therefore, the physical effective NDF (peNDF) of forage is of great importance as it provides a measure of the potential of the forage to stimulate chewing. One of the main contributing factors to peNDF is the particle size of the forage being fed. It was shown by Beauchemin et al. (1997) that short cut forages could reduce rumination time by 52 to 62% in comparison to long cut forages.

Figure 2-6 below graphically displays the relationship between rumination time and amount of peNDF contained in the diet (Zebeli *et al.*, 2010).
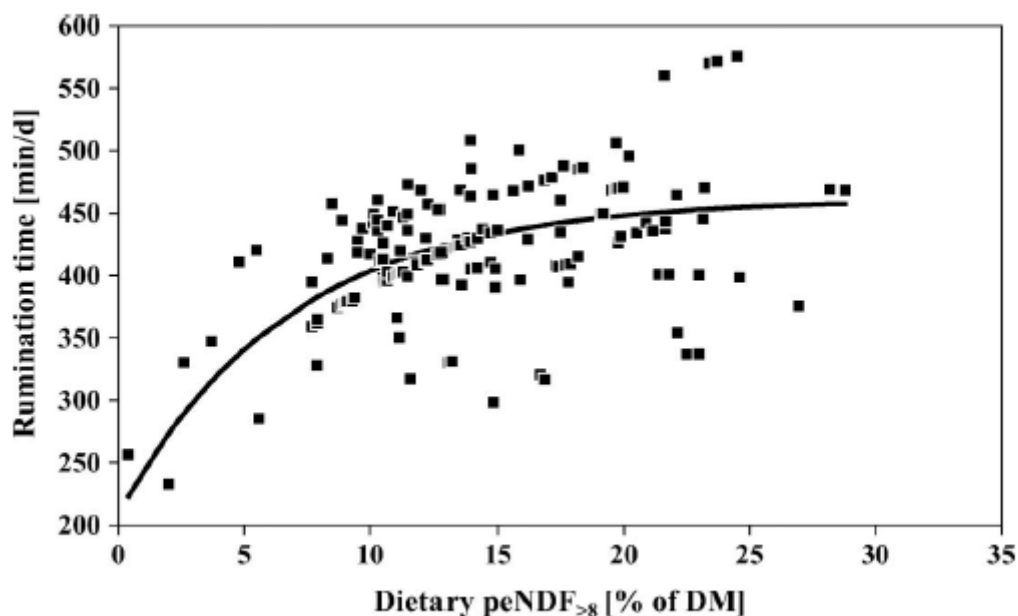
Figure 2-6. Best-fit asymptotic model showing the response of rumination time to the content of peNDF (>8mm) in the diet of dairy cows. From (Zebeli *et al.*, 2010).

2.3.4. Non-forage fibre sources

Dairy cows require forage fibre for optimal performance to maximise DMI and to stimulate chewing activity and promote fermentation (Oba and Allen, 2000a). However, as discussed previously, excess forage fibre can limit voluntary feed intake due to physical fill of the rumen. Formulating diets based on NDF as a percentage of dry matter (DM) is usually recommended because NDF has a positive relationship with rumen fill and a negative relationship with energy density (Mertens, 1994). But not all fibre comes from forage. Fibre is a part of the by-product feeds such as distiller's grains, hominy chop and maize gluten that are usually produced by extraction of starch, sugar or other valuable non-fibrous constituents (Pereira *et al.*, 1999). This is known as non-forage fibre (NFF).

Non-forage fibre has different physical and chemical properties compared to the fibre in forages (Zhu *et al.*, 1997). Diets containing higher levels of NFF are generally more easily fermentable and, to an extent, promote maximum milk production (Beauchemin and Yang, 2005) but due to their physical and chemical properties can also result in various metabolic disorders such as ruminal acidosis, milk fat depression, reduced fibre digestion, fat cow syndrome and displaced abomasum (NRC, 2001). The NDF from NFF is less effective at stimulating chewing, and consequently results in lower saliva production, than forage NDF as the particles are much smaller and therefore

ferment and pass through the rumen more quickly (Oba and Allen, 2000a). Non-forage fibre has therefore the same negative effect of reduced DMI as do the more finely chopped forages, as mentioned above in the review by Oba and Allen (2000a), due to their low peNDF. Even though these by-products contain various concentrations of NDF, they cannot be utilised as effectively as the NDF of long cut forage fibre and should thus not consist of more than 25% of the total dietary NDF (NRC, 2001).

2.4. Low crude protein diets

2.4.1. Introduction

Supplying dairy cows with the correct balance and amount of EAA in MP is necessary for maximising the efficiency of N utilisation for milk production (Ipharraguerre and Clark, 2014). Research has indicated that well fed dairy cows producing up to 30 kg of milk per day can get the majority of the AA that they require from MCP (Clark *et al.*, 1992; Nielsen *et al.*, 2003). However, as milk production increases the increasing demand for EAA needs to be gained through RUP (NRC, 2001). According to Recktenwald *et al.* (2014), CP levels of approximately 14% appeared to be approaching N deficiency for high-producing dairy cows as production began to decrease at these levels. In the rumen, proteolysis occurs faster than free AA can be utilised, resulting in higher levels of NH_3 having to be excreted. This, along with the degradation of high quality protein, has caused research to move toward finding ways to protect dietary protein from degradation in the rumen (Kamalak *et al.*, 2005). Steps that can be taken to protect proteins from rumen degradation include heat treatment, chemical treatment or modification, identification of naturally protected proteins and inhibition of proteolytic activity. By protecting protein or using protected proteins, the supply of EAA to the cow can be improved without an increase in NH_3 production (Kaufmann and Lupping, 1982).

2.4.2. Balancing forage with low protein diets

In dairy nutrition, the term "nutritional synchrony" generally refers to the provision of dietary CP in combination with energy so that they are available simultaneously for use by ruminal microbes in the correct proportions for maximised microbial production (Hall and Huntington, 2008). Although in theory nutritional synchrony should improve efficiency of nutrient use and improve production, in many studies results have shown asynchronous diets to produce similar and in some cases superior performance results for dairy cows fed in confinement (Valkeners *et al.,* 2004). Figure 2-7 illustrates theoretical rumen fermentation rates over time with proposed complementary rumen-ammonia curves (Johnson, 1976).
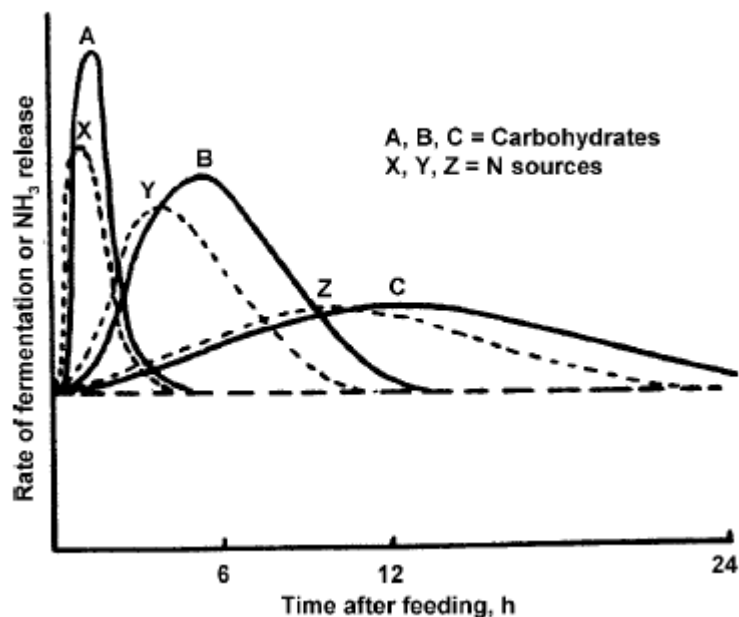
Figure 2-7. Theoretical rumen fermentation rates over time after ingestion of (A) rapidly, (B) moderately and (C) slowly fermentable carbohydrates and the proposed rumen-ammonia curves (X, Y and Z respectively) as required to support microbial protein synthesis. Adapted from (Johnson, 1976).

The efficiency with which protein is utilised in the rumen is largely dependent on the type of carbohydrate being used as an energy source. While the overall amount of N excreted by a dairy cow is directly related to the amount of N the cow consumes in its diet, the pathway through which it is excreted (urinary or faecal) is rather determined by the type of carbohydrate and type of forage (Weiss *et al.*, 2009). Also, increasing MP increases N excretion in both faeces and urine, but the increase is larger in urine. It has been suggested that MP should be balanced carefully as not to exceed the cow's requirements which would increase N excretion but at the same time not underestimate MP as this could negatively affect milk production.

In Alstrup et al. (2014) high (15.7-16%) and low (13.9-14%) CP concentrations diets were fed in conjunction with high and low organic matter (OM) digestibility. Their research showed that feed intake is affected less by the difference in protein concentration for diets with lower digestibility. For high OM digestibility, the differences between high and lower CP levels were more substantial, indicating that for better quality forages, CP level play a bigger role in DMI. Dietary CP level however did not affect intake of concentrate. They concluded that the drop in milk production from low CP concentration could not be compensated for by increased forage digestibility.

However, Hall and Huntington (2008) suggest that accurate characterisation of feed composition including protein and carbohydrate fraction and rates of fermentation, peNDF and actual microbial requirements are essential as carbohydrates vary in their fermentation characteristics. Many of these dietary parameters were not included in the study by Alstrup *et al*. (2014). Hall and Huntington concluded that interaction of microbes and diet need more attention and possibly other nutrient composition above protein and energy need to be synchronised to improve production.

In Ipharraguerre *et al*. (2005), diets with CP levels of 14, 16 and 18% were fed where the types of protein used varied to alter RUP. The other ingredients in the diet were supplied by the same source and remained constant across the protein concentrations. Their research showed very little difference in DMI and production between the different protein levels, however there was a significant effect on DMI depending on the type of protein fed.

The research from these studies suggest that low CP diets can be effective in improving NUE as long as the rest of the diet is carefully formulated to meet the microbial requirements as well as the post rumen dietary requirement of the cow.

2.4.3. Potential concerns related to low crude protein diets

Diets that are deficient in protein result in short-term effects such as limited microbial activity in the rumen. Limited microbial activity will cause a drop in daily DMI and this will ultimately result in decreased milk production (Oldham, 1984; Fisher, 2002). Dairy cows store protein in the blood, liver and muscles and these reserves can be used over short-term periods to maintain gestation and lactation. However, with normal protein turnover in the body these reserves will last long. A prolonged deficiency will have negative effects on foetus and calf growth rate. The immune system of the cow will be crippled which will result in an overall decrease in the animal's health and productive capabilities (NRC, 1978). On the other hand, when looking at the effects of protein deficient diet on reproduction, evidence suggests that excessive protein levels have a more severe effect on reproduction than diets with slight protein deficiency (Ferguson and Chalupa, 1989; Butler, 2000).

2.5. Conclusions

In the meta-analysis by Huhtanen and Hristov (2009), it was affirmed that over-feeding CP was not beneficial in improving NUE. Their review also showed that improving milk yield consequently improved NUE as long as CP wasn't increased. However, the effect of improved milk yield was less significant at improving NUE than reducing CP levels. From the findings of Weiss *et al*. (2009), it would appear that a possible way to improve NUE would be to slightly undersupply MP in

combination with a high quality forage, thus ensuring that N excretion would remain low with no proposed negative effects on production. Alstrup *et al.* (2014) however found that there was a drop in milk production for low digestible forages as opposed to high digestible forages. As our trial will be evaluating the economic feasibility of this approach to improving NUE, it will need to be investigated as to whether the decrease in feed cost due to lower CP could outweigh the possible drop in milk production. Evaluation of the direct effects of forage quality, specifically NDF digestibility, on production performance can be complex due to the need of comparing one forage type with similar NDF concentration but with differing levels of digestibility (Kendall *et al.*, 2009). There appears to be very little research that focuses on the effect that forage digestibility has on NUE and milk production and quality for varying dietary CP levels.

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Chapter 3:

Effects of forage fibre quality with varying crude protein levels on performance and nitrogen efficiency in dairy cows

Abstract

The aim of this study was to improve nitrogen use efficiency (NUE) through better nutritional management by optimizing the use of dietary protein by using better quality forages with regard to the digestibility of neutral detergent fibre (dNDF) and by reducing crude protein (CP) intake by formulating the diets to meet metabolisable protein (MP) requirements. Four lactating Holstein cows were used in a 4 x 4 Latin square design balanced for carryover effects with a 2 x 2 factorial arrangement of treatment. Treatments were low CP concentration with high NDF digestibility (**LpHd**), high CP concentration with high NDF digestibility (**HpHd**), low CP concentration with low NDF digestibility (**LpLd**) and high CP concentration with low NDF digestibility (**HpLd**). Crude protein concentrations for the rations were formulated to be around 18% for Hp and about 15% for Lp diets. The indigestible NDF, as % of the NDF, of the two oat hays used were 40.8% for Ld hay and 35.54% for the Hd hay and wheat straw was included in the Ld diets to obtain iso-NDF diets with variable quality. Cows were fed *ad libitum* for each of the 14 day treatment periods with data collection taking place over the last 4 days of each 14 day treatment period. Dry matter intake (DMI) for 3 of the diets were found to be similar, with the exception of the LpLd diet i.e. LpLd having lower DMI than the other 3 diets, showing how protein can counteract the lower forage quality by stimulating fibrolytic bacteria. Energy corrected milk yield (ECM) was found to drop 2.46kg/d for Hd forage diets and 3.00kg/d for Ld forage diets with Hp having higher production then Lp levels. Nitrogen use efficiency was found to improve by 3.04% when protein was reduced in combination with Hd forages and by 5.63% for Ld forages. We concluded that lowering protein improved NUE significantly with forage digestibility contributing to the level of improvement. However, a consequence of reducing CP was a corresponding decrease in production.

Key words: nitrogen use efficiency, milk production, environmental impact.

3.1. Introduction

The scope of dairy farming in South Africa has been changing in recent years. It is becoming more difficult for smaller scale farmers to remain competitive in the dairy industry due to increasing feed and processing costs combined with relatively small increases in producer price for milk. As a result, smaller scale farms are being sold off to larger commercial farms that can do much of their own processing on farm. Thus, there are now fewer dairy farms but much larger dairy herds per farm (Milk South Africa, 2014). Due to the increasing demand for milk and milk products, effort is being placed on increasing animal production. This increased production raises the need for nutrient rich diets that can meet the needs, particularly for energy and protein, of these high producing cows. As a result, crude protein (CP) levels in dairy rations are often as high as 18 to 20% as data published in NRC (2001) associates this increase in CP with a corresponding increase in milk production up to roughly 20% CP. This, however, results in a significant increase in nitrogen excretion with as much as 75% of the dietary N intake being excreted in the urine and faeces (Arriola Apelo *et al.*, 2014). With increasing intensification in dairy farming systems, nitrogen use efficiency (NUE) of dairy cows has gained increasing attention in recent years largely due to the environmental concerns over N emissions which are among the largest contributors to atmospheric pollution in the form of greenhouse gasses such as nitrous oxide (N_2O) (Kebreab *et al.*, 2001). Dairy farming is also considered to be the largest animal husbandry source of ammonia (NH_3) (Bussink and Oenema, 1998).

The rumen microbes utilise N to synthesise amino acids (AA) and proteins needed for microbial growth (Patton *et al.*, 2014) and in turn produce usable protein, in the form of microbial protein, for the ruminant. However, sufficient energy is needed for the microbes to efficiently utilise the N in the rumen (Oldham, 1984). The rumen microbes are able to digest large amounts of plant based material, i.e. fibre, which is indigestible to normal mammalian enzymes. They then convert it into usable energy through fermentation processes (Dewhurst *et al.*, 2000). Optimization of protein use through the use of high quality forages will assist in reducing the environmental impact of dairy farming by improving NUE (Børsting *et al.*, 2003; Yan *et al.*, 2010). With increasing prices of concentrates, forage inclusion needs to be increased. Demonstrating, therefore, how forage quality can affect efficiency with which dairy cows are able to utilise protein in their diets is of primary importance.

Studies which have looked at how forage and organic matter (OM) digestibility relate to high and low dietary CP and their effects on animal intake, production and NUE have not yet been covered very extensively in literature. In an experiment by Alstrup *et al.* (2014), they combined high (15.7 to 16.0%) and low (13.9 to14%) CP diets with high (Hd) and low (Ld) forage digestibilities. It was found that the level of CP had a more significant effect on DMI when combined with Hd forages. The conclusion of their study was that the drop in milk production from reduced DMI for low CP diets could not be compensated for by improving the forage quality. Several studies such

as Ipharraguerre *et al.* (2005), compare the effects of diets with varying CP levels (14, 16 and 18%) where the types of protein used varied to alter rumen undegradable protein (RUP). However, the other ingredients in the diet were supplied by the same source and remained constant across the protein concentrations. Thus, our research aimed to place more focus on the role of OM digestibility.

The aim of this study was to improve NUE through better nutritional management. Two different methods were combined. Firstly, optimizing the use of dietary protein using forages of different quality with regard to the digestibility of neutral detergent fibre (NDF) and then determining the effects these various qualities have on NUE. In order to achieve this, two protein levels were tested in combination with forages that displayed varying levels of NDF digestibility (NDFd). Secondly, reducing CP intake by formulating the diets to meet metabolisable protein (MP) requirements, using the latest findings in modelling, to bring about a decrease in N excretion with proposed minimal effects on milk yield and milk quality.

3.2. Materials and Methods

The trial period of 8 weeks commenced on the 22nd of January 2016 and ended on the 17th of March 2016 at Stellenbosch University's dairy research farm, Welgevallen. Adaptation to total mixed rations (TMR) diets as opposed to separate semi-complete pellets with forage (as fed on farm) commenced on the 17th of January to allow 5 additional days of adaptation to the type of diet before the adaptation period for the trial diet commenced. All procedures carried out in this experiment were approved by the Research Ethics Committee: Animal Care and Use (REC: ACU) at Stellenbosch University (protocol number SU-ACUD14-00052).

3.2.1. Animals, facilities, experimental design and feeds

Four lactating Holstein cows in second lactation were used for the trial, with average days in milk (DIM), milk yield (MY), dry matter intake (DMI) and body weight (BW) (±SD) of 214 ±17 DIM, 24.16 ± 4.92 kg/d MY, 17.8 ± 3.3 kg/d DMI and 656 ± 56 kg BW, respectively, at the beginning of the trial. Trial periods were 14 days each, with 10 days for adaptation and 4 days of data collection. Because of the small differences between treatments, adaptation periods were shorter than what usually suggested (Grant *et al.*, 2015). Grant and collaborators (2015) recently demonstrated that response to diet for eating, ruminating and resting behaviour stabilizes within 1 to 7 days, therefore an adaptation period of 7 to 14 days is sufficient for experiments investigating DMI, performance and eating behaviour, except for diets with extreme differences in their level of digestibility. Animals were housed in individual roofed stalls containing standard sized cubicles with sand bedding that

were cleaned twice daily. Each cow also had its own feed and water troughs. Cows were fed twice daily (07:00 and 17:00) after milking and had free access to clean drinking water.

The experiment was run as a 4 x 4 Latin square design balanced for carryover effects with a 2 x 2 factorial arrangement of treatments consisting of two levels of NDF quality and two levels of rumen degradable protein (RDP) (positive and negative balance) based on the latest Cornel Net Carbohydrate and Protein System (CNCPS, version 6.55) recommendations. Table 3-1 below shows the order in which the cows received the various diets over the four time periods.

Table 3-1. Experimental design of the feeding trial.

Cow	Period			
	1	2	3	4
1	A	B	C	D
2	B	C	D	A
3	C	D	A	B
4	D	A	B	C

The treatments were (A) - Low CP concentration with High NDF digestibility (**LpHd**), (B) - High CP concentration with High NDF digestibility (**HpHd**), (C) - Low CP concentration with Low NDF digestibility (**LpLd**) and (D) - High CP concentration with Low NDF digestibility (**HpLd**). Crude protein concentrations for TMR rations were formulated to be 18% for Hp diets and 15% for Lp diets. Our objective was to formulate rations similar in NDF but different in dNDF and indigestible NDF (iNDF). It was, however, difficult to isolate NDF amount from quality using only one type of forage. Our objective was therefore accomplished by including wheat straw in the Ld diets to obtain iso-NDF diets with variable quality. The iNDF, as % of the NDF, of the two oat hays used were 40.8% for Ld hay and 35.54% for the Hd hay.

The TMR diets were individually mixed in a concrete mixer each day for each animal. This was done by adding soft semi-complete pellets, that could easily disintegrate, and oat hay, that had been hammer milled through a 40mm screen, into the mixer with water added to obtain approximately 30% moisture content. Semi-complete pellets were mixed and manufactured by Afgri (Afgri Animal Feeds, Centurion, GP, South Africa). Diets were formulated to meet minimum metabolisable energy (ME) and metabolisable protein (MP) requirements and were formulated to be iso-energetic. All concentrate mixes and final diets were formulated according to the availability and chemical composition of raw ingredients as provided by Afgri Animal feeds. The difference in protein level between the Hp and Lp diets was accomplished by introducing other high CP concentration feeds, to minimize the increase in other fractions. Table 3-2 shows the raw

ingredient composition of the TMR diets and Table 3-3 shows the chemical composition of the semi-complete pellets and forages that were used in the TMR diets. All samples were analysed in triplicate.

Table 3-2. Raw ingredient composition of the semi-complete pellets provided by Afgri in g/kg as a % DM.

Feedstuff	TMR			
	LpHd	HpHd	LpLd	HpLd
Oat hay 68%	365.5	365.5	-	-
Oat hay 65%	-	-	340.1	340.1
Wheat straw	-	-	28.7	28.7
Yellow maize	254.7	244.3	251.4	241.7
Barley	92	66.7	92	66.4
Apple pomace	54	54	54	54
Molasses	11.8	12.8	11.8	12.8
Fish meal	10.8	0.5	10.8	0.5
Soya oil cake	94.2	110.3	94.2	110.2
Lupins	50.3	28.7	50.3	28.6
Gluten 21	40.5	-	40.5	-
Sunflower oil cake	-	20.7	-	20.7
Sweet lupins	-	36.2	-	36.1
Poultry by-product	-	16.4	-	16.3
Poultry blood meal	-	7.4	-	7.4
Urea	2.3	6.2	2.3	6.2
Ammonium sulphate	0.1	0.1	0.1	0.1
Salt	2.8	2.7	2.8	2.7
Megalac*	15.3	21.8	15.3	21.8
Limestone	5.5	5.5	5.5	5.5
Calcium di phosphate	0.2	0.2	0.2	0.2

*Afgri Animal Feeds, Centurion, GP, South Africa.

Table 3-3. Wet lab analysis of the chemical composition of semi-complete pellets and forages used in the TMR as % DM.

Item[1]	Feedstuff					
	LpHd	HpHd	LpLd	HpLd	Ld forage	Hd Forage
CP	15.13	18.04	14.68	17.58	4.68	5.68
Soluble protein	4.84	6.42	4.85	6.42	-	-
NDF	36.23	35.18	35.77	34.72	60.66	58.81
iNDF, % of NDF	-	-	-	-	40.80	35.54
NDFd	-	-	-	-	33.21	34.41
ADL	-	-	-	-	4.38	4.63
NFC	37.82	36.19	38.87	37.24	-	-
Sugar	5.66	5.85	4.93	5.12	-	-
Starch	25.53	23.08	25.34	22.90	5.99	5.94
Soluble fibre	6.22	6.85	3.88	4.50	-	-
EE	4.06	4.08	3.34	3.97	-	-
TFA	3.45	4.08	3.34	3.97	-	-
Ash	7.18	7.06	7.22	7.10	0.69	0.73
Ca	0.76	0.78	0.80	0.82	-	-
P	0.36	0.30	0.38	0.32	-	-
Mg	0.23	0.23	0.25	0.24	-	-
K	0.89	0.90	1.05	1.06	-	-

[1]NFC – Non-Fibre Carbohydrates; EE – Ether Extract; TFA – Trance Fatty Acids; Ca – Calcium; P – Phosphorus; Mg – Magnesium; K – Potassium; NDFd – 24 h *in vitro* NDF digestibility.

3.2.2. Sampling and Measurement

Cows were fed 105% of their voluntary daily intake during the trial. Amounts of TMR provided were adjusted throughout the trial to ensure that animals never had empty feed troughs, based on the previous day's intakes. Milk yield and BW were recorded daily using the AfiMilk dairy farming system and milk composition were monitored through the AfiFarm computer software (AfiMilk Ltd, Kibbutz Afikim, Israel). Refusals were collected and weighed daily to calculate daily feed intakes. Samples of each semi-complete pellet and oat hay were also collected on a weekly basis throughout the trial.

During the 4-day data collection phase of each of the 4 periods, samples were taken daily of TMR and refusals and were stored at -20⁰C, until analyses. Samples were then dried at 60⁰C for 72 hours in a forced air oven to determine dry matter (DM). Particle distribution for both TMR and refusals was determined using a Z-box with a 1.18 mm sieve to determine if sorting occurred. Samples were then milled in a Wiley Mill (1 mm screen; Thomas Scientific, Swedesboro, NJ). Milk samples were collected twice daily during milking (06:00 and 16:00), pooled proportionally according to morning and afternoon volumes and sent to two SANAS accredited testing laboratories, MilkoLab (GE Dairy Supplies, Parow, Cape Town, South Africa) and ARC·LNR (Agricultural Research Council, Elsenburg Analytical Services, South Africa) for fat, protein, lactose, somatic cell count (SCC) and milk urea nitrogen (MUN) analyses. Two external laboratories were used to improve accuracy and precision of final averages used and to compare the values to the on-farm AfiMilk system. Plasma, saliva, rumen fluid, urine and faeces were collected at 9-h intervals over the 4-day collection phase to get a representative of every 3 hours over a 24-hour period (02:00, 05:00, 08:00, 11:00, 14:00, 17:00, 20:00 and 23:00) and pooled at the end of each period (by cow and period). All samples were stored at -20⁰C until analyses. Faecal samples (approximately 500 g) were weighed and dried at 60⁰C for 72 hours to determine DM. After milling, faeces, TMR and refusals were analysed for DM, NDF, acid detergent lignin (ADL), iNDF, CP and starch.

Starch was determined using a modification of the acetate buffer assay as described in Hall (2008). Analyses for DM, CP and ash were performed as outlined in AOAC (2000). Indigestible NDF was determined by long term (240 hr) *in-vitro* fermentation as described in Raffrenato and Van Amburgh, (2010). Neutral detergent fibre and ADL were determined according to the procedures described in Mertens, (2002) and Raffrenato and Van Amburgh (2011), respectively.

Rumen fluid was collected using a Selekt Cattle Pump and Rumen Fluid Collector (Nimrod Veterinary Products Ltd., Moreton-in-Marsh, Gloucestershire, UK) and pH was tested immediately after collection using a handheld portable pH metre (Lasec SA, Ndabeni, Cape Town, South Africa).

Blood samples were collected in Heparin Vacutainer blood collection tubes (BD, Becton Dickinson South Africa, Woodmead, GP, South Africa) from the coccygeal vein and centrifuged within 2 hours of collection for 15 min at 3500 RPM and 4⁰C before storing.

Urine was analysed for total N, urea and creatinine. Saliva was analysed for total N and urea. Plasma was analysed for total N, AA and plasma urea nitrogen (PUN). Rumen fluid was analysed for total N, ammonia (NH_3) and volatile fatty acids (VFA). Total nitrogen was determined using the Leco Nitrogen Gas Analyser FP528 (LECO Africa (PTY) Ltd, Kempton Park, GP, South Africa). Urea, creatinine and PUN analyses were performed by IDEXX (IDEXX Laboratories PTY Ltd., Cape Town, South Africa) as well as using Ecoline® Urea test strips (DiaSys, Waterbury, Connecticut, USA). Ammonia was analysed using NH_3 slides on the IDEXX VetTest Chemistry

Analyser, using a dilution factor of 10. Volatile fatty acids were determined using a gas chromatograph (Hewlett-Packard, HP 6850) with flame ionisation detector using the procedure described by O-Thong *et al.* (2009). Amino acids were analysed at the Central analytical facility (CAF) of Stellenbosch University by means of chromatographic analysis using UPLC separation with UV or florescence detection after derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbonate (AQC).

3.2.3. Calculations and Statistical Analyses

Energy-corrected milk yield (3.14 MJ/kg) was calculated as described by Sjaunja *et al.* (1990) using the equation:

$$ECM\ (kg) = MY(kg) \times \left[\frac{38.3 \times Fat\left(\frac{g}{kg}\right) + 24.2 \times Protein\left(\frac{g}{kg}\right) + 15.71 \times Lactose\left(\frac{g}{kg}\right) + 20.7}{3.140} \right]$$

Milk nitrogen efficiency (MNE) and energy efficiency (E*eff*) were calculated as described by Phoung et al. (2013) using the equations:

$$Neff\ (\%) = \frac{MY\left(\frac{kg}{d}\right) \times N_{MILK}\left(\frac{g}{kg}\right)}{DMI\left(\frac{kg}{d}\right) \times N_{DIET}\left(\frac{g}{kg}\right)}$$

and

$$Eeff\ (\%) = \frac{MY\left(\frac{kg}{d}\right) \times E\ milk\left(\frac{Mcal}{kg}\right)}{DMI\left(\frac{kg}{d}\right) \times DE\left(\frac{Mcal}{kg}\right)}$$

With digestible energy (DE) being obtained from NDS Professional rationing system (Rum&n Sas, Reggio Emilia, Italy) and milk energy (E milk) being calculated using the formula recommended by the NRC (2001):

$$E\ milk = \left(Fat\left(\frac{kg}{kg\ milk}\right) \times 9.29\right) + \left(Protein\left(\frac{kg}{kg\ milk}\right) \times 5.47\right) + \left(Lactose\left(\frac{kg}{kg\ milk}\right) \times 3.95\right),$$

where 9.29, 5.47 and 3.95 are the amounts of energy released when 1 kg of fat, protein and lactose respectively are combusted.

Total urine excretion was estimated using creatinine as an internal marker with the standard of 0.212 mmol/kg of BW according to Chen *et al.* (1992). Total faecal matter excretion was estimated using iNDF as an internal marker, after apparent total tract digestibility was calculated. Total excretion of faeces and urine were then used to determine total N excreted (kg/d). The total NUE was determined using the relationships between N intake, N excretion and milk N.

Experimental data were analysed using the GLIMMIX procedure of SAS software (Version 9.4, 2013; SAS Institute Inc., Cary, NC). All response variables were analysed with period and treatment as fixed factors and cow as random factor. Differences between treatments and periods were determined by least significant difference method with a Tukey adjustment. Statistical differences were considered significant at $P \leq 0.05$ and those between $0.05 < P \leq 0.10$ were considered trends. Results reported in tables are, if not otherwise indicated, treatment least square means (LSM) and respective standard errors (SEM).

3.3. Results and Discussion

As previously described, the 4 concentrates of the final diets were formulated based on the nutritional compositions provided by Afgri, as ingredients were not available to be individually analysed in the laboratory. After the mixed concentrates samples were analysed, it was however found that both CP and NDF levels and quality differed to those expected. These discrepancies were most likely due to different sources of certain ingredients used during the manufacturing process (the forage-free mixes were provided in pellets) in the feeds at the production plant which differed from the records we used. Another possibility is also given by the fact that most feed companies in South Africa use near infrared analysis (NIR) instead of wet chemistry (supposedly more accurate and precise, as opposed to NIR which is heavily dependent on frequent calibrations with large data sets of local feeds) as used in our laboratory. Nutritional compositions of the four diets provided to the cows are reported in Table 3-4.

Table 3-4. Chemical composition of TMR diets fed to the animals as % DM

Item[1]	Diets			
	LpHd	**HpHd**	**LpLd**	**HpLd**
NDF, % of DM	34.12	32.83	33.34	32.02
iNDF, % of NDF	31.80	36.06	31.57	35.61
ADL, % of DM	2.69	2.06	2.13	2.33
CP, % of DM	14.41	16.44	14.75	17.49
Starch, % of DM	24.69	24.56	25.29	25.29
Ash, % of DM	1.26	1.29	1.38	1.12

[1]CP – Crude Protein; NDF – Neutral Detergent Fibre; iNDF – indigestible NDF; ADL – Acid Detergent Lignin

3.3.1. Feed intake and efficiency

Refusal and TMR particle distributions were analysed showing no significant sorting of diets ($P = 0.45$) across periods. Diet had a significant effect ($P < 0.0001$) on DMI, however the LpLd diet was the only diet considered significantly lower than the other 3 diets. Results were similar for DMI as a percentage of BW as shown in Table 3-5 below. These results correspond to the findings of Weisbjerg *et al.* (2010) who concluded that cows receiving better quality forages are less sensitive to low CP levels than those on low quality forages. In both analyses, however, it was noted that the Ld diets had numerically lower DMI than the Hd diets for the same protein level.

Diet also had a significant effect ($P = 0.0009$) on E*eff*. The Ld diets presented no difference to each other whereas the Hd diets and the HpLd diet were presented as similar. Even though HpLd resulted statistically similar to the Hd diets, we can notice the trend ($P = 0.10$) of the HpLd resulting in higher E*eff* than the Hd diets.

Table 3-5. Least squares mean of dry matter intakes and energy efficiencies of the 4 diets and respective pooled standard errors and significance levels of differences between treatments.

Item	Diet				SEM	*P*
	LpHd	**HpHd**	**LpLd**	**HpLd**		
DMI, kg/d	17.80[a]	18.47[a]	16.13[b]	17.75[a]	0.312	< 0.0001
DMI, % of BW	2.83[a]	2.79[a]	2.43[b]	2.69[a]	0.050	< 0.0001
E*eff*, %	51.24[b]	51.39[b]	57.91[a]	55.48[ab]	0.018	0.0009

[ab] Means within a row not sharing the same superscript differ (*P* < 0.05)

3.3.2. Milk yield and composition

Diet proved to have a significant effect for both milk yield (*P* < 0.0001) and ECM (*P* < 0.0001) with Hp diets resulting in higher milk production than Lp diets with no difference between Hd and Ld forages. For milk quality, milk samples were analysed at two external laboratories to improve precision and accuracy of result. The averages for these analyses were then gained and used in the statistical model. Milk analyses from both companies are provided in Appendix A.

When looking at fat and protein content as percentages, despite diet presenting statistical differences for fat (*P* = 0.0046) and protein (*P* = 0.0001) we see that no connections between protein level and dNDF are statistically identifiable under the Tukey comparison for least significant differences and the numerical differences between treatments are too low to conclude that the results are biologically justified by the diets. For fat, the Ld diets have the higher numerical values with HpLd being notably higher than the rest. A possible reason for this is that the added straw in the Ld diets may have decreased the passage rate and therefore lower amounts of biohydrogenation intermediates, having anti-lipogenic effects, might have reached the small intestine resulting in higher fat percentage (Bauman and Griinari, 2003). However, fat, protein and lactose volumes (g/d) follow the trend of milk yield as they are a function of milk production showing results with significant effects between Hp and Lp diets with no significant effect from forage digestibility. Table 3-6 below shows the results for milk yield and quality.

Table 3-6. Least squares mean of milk production and quality as a function of production volume of the 4 diets and respective pooled standard errors and significance level of differences between treatments.

Item	Diet				SEM	*P*
	LpHd	HpHd	LpLd	HpLd		
MY, kg/d	24.94[b]	26.94[a]	23.99[b]	26.81[a]	0.291	< 0.0001
ECM, kg/d	23.44[b]	25.90[a]	23.36[b]	26.36[a]	0.330	< 0.0001
Fat, %	3.06[b]	3.27[ab]	3.30[ab]	3.42[a]	0.067	0.0046
Protein, %	3.22[ab]	3.14[c]	3.26[a]	3.19[bc]	0.014	< 0.0001
Lactose, %	4.67	4.64	4.62	4.68	0.018	0.5922
Fat, g/d	756.62[b]	871.92[a]	789.06[b]	908.14[a]	19.287	< 0.0001
Protein, g/d	797.98[b]	844.04[a]	770.73[b]	848.90[a]	10.270	< 0.0001
Lactose, g/d	1166.94[b]	1249.56[a]	1112.23[b]	1257.80[a]	14.705	< 0.0001
SSC, ×10^3/ml	112.16[a]	105.31[a]	248.25[b]	103.59[a]	30.555	0.0029

[ab] Means within a row not sharing the same superscript differ ($P < 0.05$)

Somatic cell counts (SCC) were affected by diet with the LpLd treatment resulting in the highest level. However, raw data show an unusually high spike in SSC for one animal over 2 days of the entire trial period. The cause of the spike is uncertain but it is very unlikely that it is due to the diet and with the two outlying data points removed from the analysis no significant difference between diets is noted. Therefore, we do not consider diet to have a significant effect to the SCC.

3.3.3. Nitrogen intake, nitrogen excretion, milk nitrogen and nitrogen efficiency

Daily nitrogen intakes were calculated from DMI and diets' CP, assuming homogenous distribution between offered diets and refusals. Amounts of protein ingested resulted in Hp diets having similar total N intake which were higher than that of both the Lp diets of which the Hd diet had a statistically higher N intake than the Ld diet. The reason for this difference between the Lp diets is that diet LpLd had significantly lower DMI than the other 3 diets (Table 3-5).

When Looking at the Tukey comparison for N excreted in faecal matter, a trend ($0.05 < P < 0.10$) can be identified for the Hp diets producing slightly higher levels of N excretion than for LpLd. However, N excreted in faecal matter was considered similar across all diets ($P = 0.2101$) which is expected as faecal N has more stability as it is mostly undigested feed N or microbial N (Van Horn *et al.*, 1994). Nitrogen excreted in urine however showed a significant difference for diet ($P = 0.0003$) with increased N excretion for the Hp diets which is consistent with the findings of Marini and Van Amburgh (2005). Forage digestibility had a significant effect on urinary N for the Hp diets but not for Lp diets, with Hd diets resulting in lower levels of N excretion than Ld diets. These results are expected as excess N favours the path of urinary excretion (Marini and Van Amburgh, 2005) with faecal excretion remaining fairly constant (Van Horn *et al.*, 1994). Excess N exists in various forms and this form is what determines its excretory pathway. In the rumen, NH_3 is produced from deamination of AA and peptides and from dietary NPN sources. Most of the excess NH_3 is absorbed through the rumen wall into the blood stream. Ammonia is however toxic to the animal so it needs to be transported to the liver where it is converted to urea (Lapierre *et al.*, 2005) from where it can be recycled or excreted. This formation of urea in the liver is however very costly to the animal in terms of energy and uses energy from the diet or body tissue, depending on the physiological phase of the cow. Therefore, excess N needs to be kept to a minimum to prevent this waste of energy. This finding for urinary N is consistent with the results for PUN which show higher levels for Hp diets with PUN levels for HpLd being significantly higher than those of HpHd. This shows that high CP diets result in higher levels of ammonia production and N excretion in the urine. It also suggests that the rate at which microbes can utilise the ammonia is affected by the quality of the forage for higher CP levels. The most likely reason for the more significant role of forage digestibility on higher CP diets is that of the extra energy that can be derived through more complete fermentation of the Hd forages allowing the microbes to better utilise the inorganic N in the rumen (Oldham, 1984; Dewhurst *et al.*, 2000). Milk urea nitrogen (MUN) showed higher levels for Hp diet than Lp diet but there was no significant difference between forage levels for the Lp diets, forage digestibility appears to have a significant effect on MUN for Hp diets with Ld forage resulting in significantly higher levels of MUN than Hd forages.

Diet had a significant effect ($P < 0.0001$) on total milk N with Hp diets having higher levels than Lp diets. Diet also had a significant effect ($P < 0.0001$) on MNE with the Hp diets producing lower MNE than the Lp diets with no significant difference between forage digestibility. Results of N fractions and MNE are shown below in Table 3-7 with the relationship between the results of excess N depicted in Figure 3-1.

Table 3-7. Nitrogen intake, nitrogen excreted and milk urea nitrogen for the four treatments.

Item	Diet				SEM	*P*
	LpHd	HpHd	LpLd	HpLd		
Intake, g/d	410.36[b]	487.39[a]	380.04[c]	496.87[a]	7.882	< 0.0001
Faecal, g/d	155.90[a]	174.51[a]	134.62[a]	167.61[a]	17.813	0.2101
Urinary, g/d	159.93[c]	201.67[b]	156.46[C]	267.28[a]	11.638	0.0003
Milk, g/d	125.08[b]	132.29[a]	120.80[b]	133.06[a]	1.610	< 0.0001
Excreted, g/d	315.83[c]	376.18[b]	291.07[c]	434.89[a]	20.598	0.0005
MUN, mg/dl	14.13[c]	17.32[b]	14.96[c]	20.22[a]	0.476	< 0.0001
PUN, mmol/L	4.925[c]	6.600[b]	5.225[c]	8.000[a]	0.327	< 0.0001
MNE, %	30.55[a]	27.51[b]	32.37[a]	26.74[b]	0.007	< 0.0001

[ab] Means within a row not sharing the same superscript differ ($P < 0.05$)

In Figure 3-1 it can be seen that Hp diets resulted in higher total volumes of N being excreted when compared to Lp diets. Figure 3-2 shows what percentage of the total N excreted, is excreted via each excretory pathway. It can also be seen that the differences in N excreted via faeces are numerically similar (as a percentage of total N excreted) especially within the respective fibre qualities. However, far larger variances can be seen when comparing urinary N excretions with Hp diets producing higher levels of N excretion. This indicates that Hp diets result in much higher amounts of urea being produced by the liver, above that which can be recycled by the animal, thus increasing the amount that needs to be excreted.
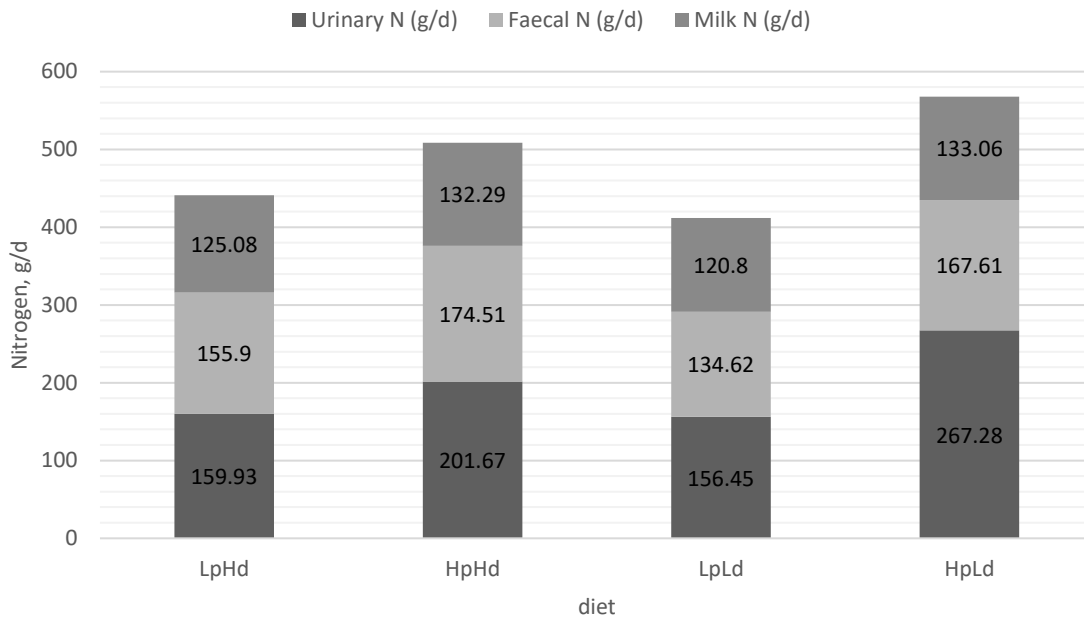
Figure 3-1. Nitrogen output pathways across treatments.
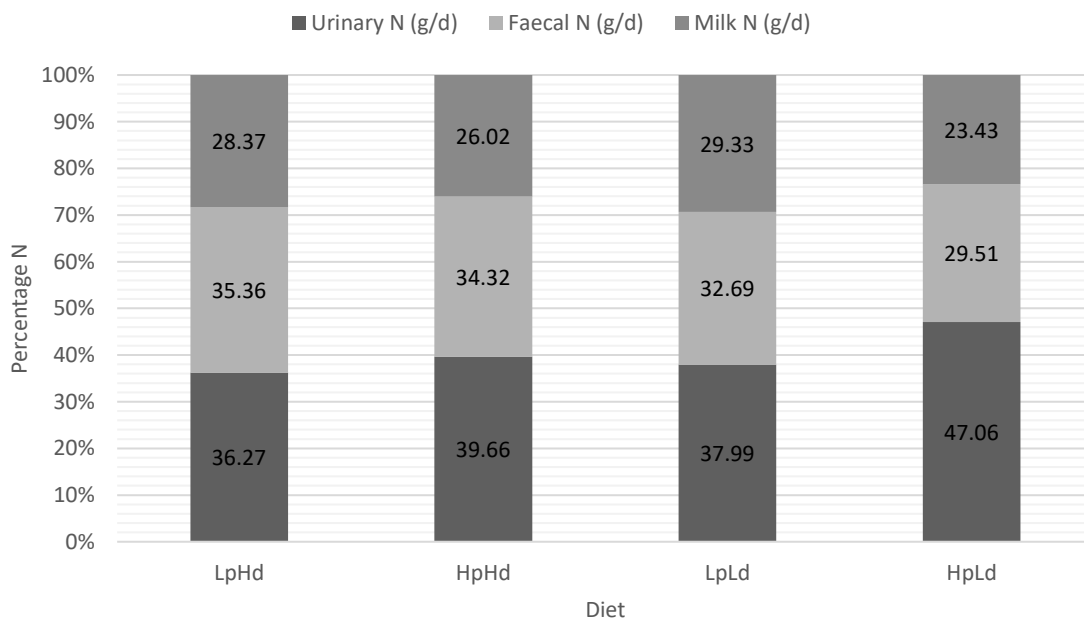
Figure 3-2. Nitrogen output pathways across treatments as % of total N.

Rumen pH, ranging between 6.42 and 6.89 for all animals throughout the trial, was analysed in SAS and presented no significant difference between treatments. Volatile fatty acid concentrations were also analysed in SAS but no statistical differences were found between treatments either with an average of 29.9 mg/dL acetate, 16.39 mg/dL propionate and 7.95 mg/dL butyrate. This suggested that the rumen microbial population adapts to the various diets to maintain a relatively constant environment which is expected for diets that do not differ vastly in starch and NDF levels like ours. Similarly, AA concentrations in the plasma were analysed but no significant differences were noted. This shows that the consistency of the proteins in the blood stream was not affected by the diet, but only the levels of urea being transported to the kidneys for excretion.

3.4. Conclusions

The main objective of our experiment was to demonstrate the role that forage quality (i.e. NDFd and iNDF) can play, when lowering protein content of the diet for lactating dairy cows. However, our attempt was biased by logistics that resulted in very low differences in terms of fibre quality and protein of the specific diets. Also, because of the low forage quality, for both hays used, forage inclusion could not be higher than 40% to allow the desired metabolisable energy. Higher forage inclusion may have resulted in larger differences. To increase difference in fibre quality, between higher and lower digestibility treatments, wheat straw was included to the amount of 2.9% of the pellet. The presence of straw probably resulted in decreased intake for the Ld diets, the difference between forage digestibility was significant only for the lower CP diet (i.e. LpLd) showing how protein can counteract the lower forage quality, by stimulating fibrolytic bacteria. This is confirmed by the fact that the same diet resulted in the highest energy efficiency diet. Due to the lower than expected CP levels in the Lp diets, the Hp diets resulted in the highest milk yield and quality and therefore no conclusions can be done in terms of higher quality forage and lower dietary protein. It was however demonstrated that reducing CP significantly improves (P < 0.0001) MNE and reduces the total volume of N excreted (P = 0.0005) into the environment with a reduction of 60.35 g/d of N (376.18 (Hp) to 315.83 (Lp)) on Hd forage diets and a reduction of 143.82 g/d of N (434.89 (Hp) to 291.07 (Lp)) on Ld forage diets (Table 3-7). Nitrogen use efficiency of the LpLd treatment was 32% which is higher than average for intensive dairy systems (Lapierre and Lobley, 2001; Ipharraguerre et al., 2005), showing how N use can be greatly improved and could subsequently result in lower costs per unit of milk produced. This research will prove useful when regulations regarding N excretions are introduced in South Africa as they have been in many European Union (EU) countries.

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Chapter 4:

Investigating the economics of feeding low crude protein diets in dairy cows

Abstract

The aim of this chapter was to investigate the possible economic benefits for dairy farmers presented through better nutritional management optimizing the use of dietary protein by using better quality forages with regard to the digestibility of neutral detergent fibre (dNDF) and by reducing crude protein (CP) intake by formulating the diets to meet metabolisable protein (MP) requirements. Four lactating Holstein cows were used in a 4 x 4 Latin square design balanced for carryover effects with a 2 x 2 factorial arrangement of treatment. Treatments were low CP concentration with high NDF digestibility (**LpHd**), high CP concentration with high NDF digestibility (**HpHd**), low CP concentration with low NDF digestibility (**LpLd**) and high CP concentration with low NDF digestibility (**HpLd**). Crude protein concentrations for the rations were formulated to be around 18% for Hp and about 15% for Lp. The indigestible NDF, as % of the NDF, of the two oat hays used were 40.8% for Ld hay and 35.54% for the Hd hay and wheat straw was included in the Ld diets to obtain iso-NDF diets with variable quality. Cows were fed *ad libitum* for 14 days with data collection taking place over the last 4 days of each period. Daily dry matter intakes (DMI) and milk yields were recorded and used to determine daily feed costs and income respectively. These were then used to calculate income over feed cost (IOFC). It was seen the higher protein diets had a higher cost per day but also resulted in higher milk production. The impact of better quality forages can also clearly be seen, especially on the lower protein levels. Statistically diet had no effect on IOFC, with IOFC being the same across all treatments. However, IOFC is the result of a combination of feed cost and income therefore having a higher standard error of the mean (SEM) than the other two factors. Numerical differences that would be significant on farm level were however observed and we concluded that it would be of interest to perform this study on a larger herd to improve statistical variation.

Key words: Income over feed cost, milk production, feed cost, nutritional management.

4.1. Introduction

The scope of dairy farming in South Africa has been changing in recent years. It is becoming more difficult for smaller scale farmers to remain competitive in the dairy industry due to increasing feed and processing costs combined with relatively small increases in milk price. As a result, smaller scale farms are being sold off to larger commercial farms that can do much of their own processing on farm and dilute fixed costs. Thus, there are now fewer dairy farms but much larger dairy herds per farm (Milk South Africa, 2014). Due to the increasing demand for milk and milk products, effort is being placed on increasing animal production. This increased production raises the need for nutrient rich diets that can meet the requirements, particularly for energy and protein, of these high producing cows. As a result, crude protein (CP) levels in dairy rations are often as high as 18 to 20% as data published in NRC (2001). The NRC associates this increase in CP with a corresponding increase in milk production up to roughly 20% CP.

With increasing intensification in dairy farming systems, nitrogen use efficiency (NUE) of dairy cows has gained increasing attention in recent years largely due to the environmental concerns over N emissions and to increased costs of protein sources. Nitrogen use efficiency is an expression of the ratio between N used to synthesise milk protein and the dietary N intake (Higgs *et al.*, 2013). This efficiency of N use for dairy production systems rarely exceeds 25 to 30%, i.e. approximately 70% of ingested N is excreted into the environment (Lapierre and Lobley, 2001; Ipharraguerre *et al.*, 2005) with approximately 25% of the N being converted into milk (Arriola Apelo *et al.*, 2014).

In Shalloo *et al.* (2004) it was demonstrated that feed costs contribute to more than 50% of total dairy production costs with protein being one of the most expensive feed components. Verbal communication with some of the Western Cape dairy farmers farming with intensive confinement systems using finely tuned TMR rations reported that feed cost can reach as much as 75% of their total production costs. With current feed prices still on the rise, particularly those used in concentrate feeds, interest in substituting energy-rich grains with high-quality forages and optimizing the use of dietary protein sources is growing rapidly (Hymøller *et al.*, 2014). One way to achieve better NUE is to reduce dietary CP intake (Nielsen *et al.*, 2003; Kalscheur *et al.*, 2006) which subsequently reduces the excreted N levels (Børsting *et al.*, 2003) and will result in lower cost diets. However, reducing CP intake in high producing dairy cows can run the risk of negative effects on dry matter intake (DMI) and this in turn will reduce milk production (Fisher, 2002; Hristov *et al.,* 2005) with subsequent loss of income. Therefore, care must be taken in balancing diets, while lowering protein concentration, to prevent a decrease in DMI.

Considering what has already been discovered with regard to the interaction between CP levels and CP quality (or type i.e. RUP or RDP) and forage digestibility, the aim of this study was to improve NUE through better nutritional management. A secondary objective of the study was to

look at the possible economic implications that improved NUE, with lower CP levels, may present to the farm's income over feed costs.

4.2. Materials and Methods

The trial period of 8 weeks commenced on the 22nd of January 2016 and ended on the 17th of March 2016 at Stellenbosch University's dairy research farm, Welgevallen. All procedures carried out in this experiment were approved by the Research Ethics Committee: Animal Care and Use (REC: ACU) at Stellenbosch University (protocol number SU-ACUD14-00052).

3.2.1. Animals, facilities, experimental design and feeds

Four lactating Holstein cows were used for the trial, with average days in milk (DIM), milk yield (MY), dry matter intake (DMI) and body weight (BW) (±SD) of 214 ±17 DIM, 24.16 ± 4.92 kg/d MY, 17.8 ± 3.3 kg/d DMI and 656 ± 56 kg BW, at the beginning of the trial. Trial periods were 14 days each with 10 days for adaptation and 4 days of data collection. Because of the small differences between treatments, adaptation periods were shorter than usually suggested. Grant and collaborators (2015) recently demonstrated that response to diet for eating, ruminating and resting behaviour stabilizes within 1 to 7 days, therefore an adaptation period of 7 to 14 days is sufficient for experiments investigating DMI, performance and eating behaviour, except for diets with extreme differences in their level of digestibility. Animals were housed in individual roofed stalls containing cubicles with sand bedding that were cleaned twice daily. Each cow had its own feed and water trough. Cows were fed twice daily (07:00 and 17:00) after milking and had free access to clean drinking water.

The experiment was a 4 x 4 Latin square design balanced for carryover effects with a 2 x 2 factorial arrangement of treatments consisting of two levels of NDF quality and two levels of rumen degradable protein (RDP) (positive and negative balance) based on the latest Cornel Net Carbohydrate and Protein System (CNCPS, version 6.55) fractionation system (Van Amburgh *et al.*, 2015).

The treatments were (A) - Low CP concentration with High NDF digestibility (**LpHd**), (B) - High CP concentration with High NDF digestibility (**HpHd**), (C) - Low CP concentration with Low NDF digestibility (**LpLd**) and (D) - High CP concentration with Low NDF digestibility (**HpLd**). Crude protein concentrations for TMR rations were formulated to be 18% for Hp and15% for Lp. Our objective was to formulate rations similar in NDF but different in dNDF. It was, however, difficult to isolate NDF amount from quality using only one type of forage. Therefore, wheat straw was including in the Ld diets to obtain iso-NDF diets with variable quality. The iNDF, as % of the NDF, of the two oat hays used were 40.8% for Ld hay and 35.54% for the Hd hay.

All diets were formulated according to the raw ingredient composition and availability as provided by Afgri Animal feeds. Individual daily feed intakes (on as-fed basis) were determined after cows were fed 105% of their voluntary daily intake during the trial. Amounts of TMR provided were adjusted throughout the trial to ensure that animals never had empty feed troughs, based on the previous day's intakes. Table 4-1 below shows the list of ingredients and how much of each in grams per kilogram (g/kg) was used in the concentrate for each of the four diets.

Table 4-1. Raw ingredients of the 4 pellets provided by Afgri in g/kg.

Feedstuff	Diets*			
	LpHd (A)	HpHd (B)	LpLd (C)	HpLd (D)
Oat hay 68%	365.6	365.5	-	-
Oat hay 65%	-	-	340.1	340.1
Wheat straw	-	-	28.7	28.7
Yellow maize	254.7	244.3	251.4	238.1
Barley	92	66.7	92	66.4
Apple pomace	54	54	54	54
Molasses	11.8	12.8	11.8	12.8
Fish meal	10.8	0.5	10.8	0.5
Soya oil cake	94.2	110.3	94.2	110.2
Lupins	50.3	28.7	50.3	28.6
Gluten 21	40.5	-	40.5	-
Sunflower oil cake	-	20.7	-	20.7
Sweet Lupins	-	36.2	-	36.1
Poultry by-product	-	16.4	-	16.3
Poultry blood meal	-	7.4	-	7.4
Urea	2.3	6.2	2.3	6.2
Ammonium sulphate	0.1	0.1	0.1	0.1
Salt	2.8	2.7	2.8	2.7
Megalac*	15.3	21.8	15.3	21.8
Limestone	5.5	5.5	5.5	5.5
Calcium di phosphate	0.2	0.2	0.2	0.2

* Afgri Animal Feeds, Centurion, GP, South Africa

3.2.2. Measurements and calculations

Current pricing (May 2016) of raw ingredients was obtained from Afgri Animal Feeds and is shown in Table 4-2. Cost of feed per tonne was calculated using the CNCPS based rationing software NDS Professional (Rum&n Sas, Reggio Emilia, Italy) which was also used to formulate the diets.

Table 4-2. Raw feed ingredient cost supplied by Afgri in South African Rand per tonne.

Feedstuff	Cost*
Oat hay 68%	R 2 000.00
Oat hay 65%	R 2 000.00
Wheat straw	R 1 200.00
Yellow maize	R 3 741.00
Barley	R 3 742.00
Apple pomace	R 1 800.00
Molasses	R 3 450.00
Fish meal	R 17 000.00
Soya oil cake	R 8 025.00
Lupins	R 3 400.00
Gluten 21	R 3 555.00
Sunflower oil cake	R 4 600.00
Sweet Lupins	R 3 400.00
Poultry by product	R 6 000.00
Poultry blood meal	R 14 000.00
Urea	R 4 590.00
Ammonium sulphate	R 1 900.00
Salt	R 680.00
Megalac*	R 11 047.00
Limestone	R 517.00
Calcium di phosphate	R 9 880.00

* Afgri Animal Feeds, Centurion, GP, South Africa

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Milk yield was recorded daily using the AfiMilk dairy farming system and milk composition and animal activity were monitored through the Afifarm computer software (AfiMilk Ltd, Kibbutz Afikim, Israel). Refusals were collected and weighed daily to record daily feed intake from which daily DMI was calculated. Daily feed intakes and daily milk productions averages were calculated per diet across the entire trial. Current milk price was obtained from industry representatives. Only milk yield was used for this calculation without regard to milk quality parameters as milk is not paid for according to quality.

Experimental data were analysed using the GLIMMIX procedure of SAS software (Version 9.4, 2013; SAS Institute Inc., Cary, NC). All response variables were analysed with period and treatment as fixed factors and cow as random factor. Differences between treatments and periods were determined by least significant difference method with a Tukey adjustment. Statistical differences were considered significant at $P \leq 0.05$ and those between $0.05 < P \leq 0.10$ were considered trends. Results reported in tables are, if not otherwise indicated, treatment least square means (LSM) and respective standard errors (SEM).

4.3. Results and Discussion

The feeds were formulated based on the nutritional compositions provided by Afgri, as ingredients were not available to be analysed individually in the laboratory. After the mixed semi-complete pellet samples were analysed, it was found that both CP and NDF levels and quality differed slightly to those expected. These discrepancies are most likely due to different sources of certain raw ingredients used during the manufacturing process of the pellets at the production plant, which differed from the records we used.

Dry matter intakes were recorded and results varied between 16.13 and 18.47 kg/d for all diets. Feed prices were calculated within the NDS Professional rationing system and based on pricing obtained from Afgri Animal Feeds and current oat hay prices (May 2016). Diet costs are shown in Table 4-3 below. Oat hay cost is not based on quality but price per bale is fixed with bales considered to average 25 kg per bale.

Table 4-3. TMR prices per tonne.

Feed	Cost	
LpHd	R 3 579.17	/ tonne
HpHd	R 3 749.75	/ tonne
LpLd	R 3 655.47	/ tonne
HpLd	R 3 837.07	/ tonne

Total daily feed costs were calculated based on daily intakes of respective diets on DM basis. Milk income per day was calculated based on the current (May 2016) milk price of R 5.00 per litre using daily milk yield per day (MY/d) on each diet.

When we consider the Tukey grouping of diet LSM for feed cost (Table 4-4), we can see that the Hp diets have no statistical difference (P = 0.8398). Although LpHd and HpLd are considered to have no difference under the given criteria, there is a trend (P =0.0545) toward them differing and it is likely that a larger data pool would produce more definitive results. The LpLd diet was shown to have the lowest cost per day. The most likely reason it differed so significantly from LpHd in price (P = 0.0235) was due to the inclusion of wheat straw in the pellet replacing a portion of the more expensive oat hay. Along with the price difference, cost per day was based on daily DMI and the LpLd diet had significantly lower intake than the other diets which was likely due to inclusion of straw which reduced passage rate.

When considering income, diet can be seen to have an effect (P = 0.0001) with the Tukey grouping of diet LSM for income showing the Hp diets grouped together with better income i.e. higher milk production levels, and the Lp diets grouped together (Table 4-4). Although statistically similar, numerically a difference can be noticed between LpHd and LpLp. This numerical difference is not present for the Hp diets. A larger data pool may also produce more definitive results in this instance.

Table 4-4. Least squares mean of feed cost, income and IOFC and the respective pooled standard errors and significance levels of differences between treatments.

Item*	Diet				SEM	*P*
	LpHd	**HpHd**	**LpLd**	**HpLd**		
Feed Cost /d	63.76[b]	69.40[a]	58.91[c]	68.05[ab]	1.6335	< 0.0001
Income /d	121.06[b]	130.76[a]	116.47[b]	130.16[a]	1.9949	< 0.0001
IOFC /d	57.30[a]	61.36[a]	57.56[a]	62.11[a]	2.5893	0.1462

*All values in South African Rand
[ab] Means within a row not sharing the same superscript differ (*P* < 0.05)

Income over feed cost was calculated for each diet using daily income and daily feed costs and results in LSM are compared in Figure 4-1 between Hp and Lp diets for both Hd and Ld forages.
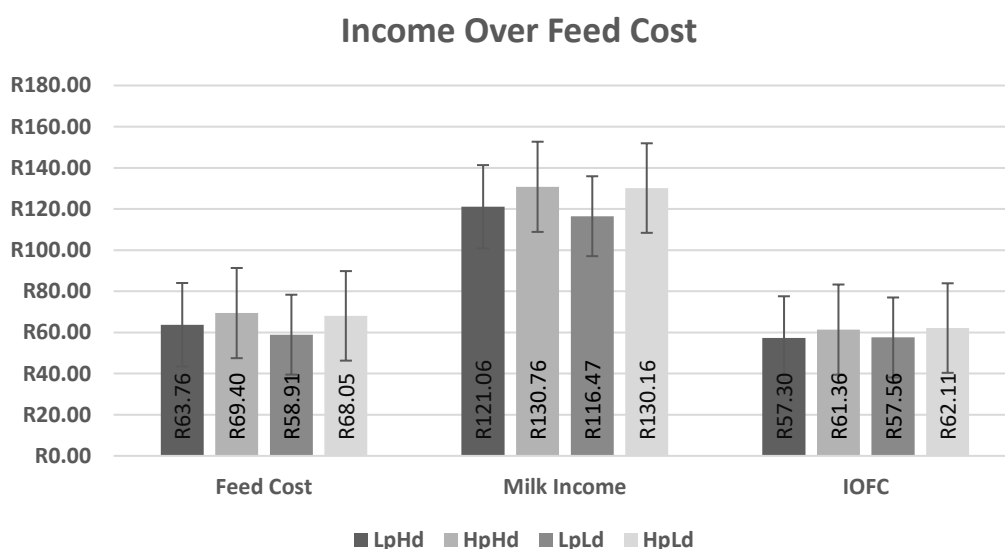
Figure 4-1. Income over feed cost for each treatment (LSM).

Statistically, diet had no effect (*P* = 0.1462) on IOFC with the Tukey grouping affirming this result (Table 4-4). Income over feed cost is the result of a combination of feed cost and income therefore having a higher SEM than the other two factors. This is the most probably reason for the IOFC being statistically similar and a larger experimental group may be needed to identify a difference in IOFC between the various diets as numerical differences can currently be observed.

The monetary difference between Hp and Lp diets were also determined using IOFC for the diets and a range of R 3.80 to R 4.81 of increased income using Hp diets was noted which on a farm level would be considered a very significant amount when expressed over the entire herd.

The monetary difference in cost of feed and income from milk between Hp and Lp diets was also determined using the average of daily values per treatment with a difference of between R 4.29 to R 10.49 being obtained for feed cost and a difference between R 9.10 to R 14.29 for income. These values suggest that the economic benefits of reduced protein on feed cost do not make up for the reduced income resulting from the drop in milk production on Lp diets. Although both Lp diets resulted in reduced income (i.e. lower milk production), it can be seen that losses for Hd forage diets (R9.41/d) are smaller in comparison to the losses that arise from Ld forage diets (13.99/d).

4.4. Conclusions

In this study, it was seen that lowering CP did not provide economic benefit for the farmer which would most likely result in farmers opting to continue using Hp diets to maintain high production. Milk prices however are based on yield only, without consideration being given to composition. Diet was shown to have an effect on milk fat % (P = 0.0046) which is the qualitative feature by which milk is most commonly appraised (MilkSA). However, this rating of milk quality is more relevant to Jersey herds which naturally produce higher levels of butter fat for which a premium I received. Thus, it may be of interest to perform this experiment on breeds such as the Jersey to determine if there may be more significant economic benefits to these farmers.

The impact of better quality forages can however clearly be seen despite the differences being smaller than desired. It should be noted that forage sourcing for this trial took place during a time of extreme drought in South Africa meaning forage quality in general was very poor, even for the Hd forages. This resulted in diets with high indigestible fibre content, lower than expected dNDF, resulting in lower nutrient density and, likely, in higher filling effect. This could explain the low DMI (16.13 to 18.47 kg/d) and its resulting decreased milk production as recorded in this research experiment in comparison to those of Alstrup *et al.* (2014) which recorded a minimum of 21.9 kg/d DMI, which is also consistent with other studies that were reviewed (Lee *et al.*, 2012; Giallongo *et al.*, 2015). A repeat of this study, utilising better quality forages with the objective of increasing DMI, could very likely prove to have an economic benefit to farmers. Thus, further research utilising a larger sample group of cows and feeding forages with a more pronounced difference in quality is strongly suggested.

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Chapter 5:
General conclusions

The primary objective of our experiment was to demonstrate the effect of forage fibre quality (i.e. NDFd and iNDF) on the efficiency with which dairy cows are able to utilise protein in their diets and addressing the environmental factors involved in dairy feed management through the principle of improved NUE. To determine the effects, we combined two different methods. Firstly, optimizing the use of dietary protein using forages of different quality with regard to the digestibility of NDF and then determining the effects these various qualities have on NUE. Two protein levels were tested in combination with forages that displayed varying levels of dNDF. Secondly by reducing CP intake by instead formulating the diets to meet MP requirements to bring about a decrease in N excretion with proposed minimal effects on milk yield and milk quality. The secondary objective was to determine if lower CP diets with better quality forages presented any economic benefits to the farmer due to reduced feed cost. As our trial was evaluating the economic feasibility of this approach to improving NUE, we needed to investigate whether the decrease in feed cost due to lower CP could outweigh the possible drop in milk production. Evaluation of the direct effects of forage quality, specifically NDF digestibility, on production performance were complex due to the need of comparing one forage type with similar NDF concentration but with differing levels of digestibility (Kendall *et al.*, 2009).

It was demonstrated that reducing CP significantly improves ($P < 0.0001$) MNE and reduces the total volume of N excreted ($P = 0.0005$) into the environment with a reduction of 60.35 g/d of N (376.18 (Hp) to 315.83 (Lp)) on Hd forage diets and a reduction of 143.82 g/d of N (434.89 (Hp) to 291.07 (Lp)) on Ld forage diets (Table 3-7). Nitrogen use efficiency of the LpLd treatment was 32% which is higher than average for intensive dairy systems (Lapierre and Lobley, 2001; Ipharraguerre *et al.*, 2005), showing how N use can be greatly improved and could subsequently result in lower costs per unit of milk produced. This research will prove useful when regulations regarding N excretions are introduced in South Africa as they have been in many European Union (EU) countries.

However, these results came at the cost of a drop in 2L/d of milk for Hd forages and a drop of 2.82L/d for Ld forage diets. It was observed that lowering CP does not provide economic benefit for the farmer which would most likely result in farmers opting to continue using Hp diets. The impact of better quality forages can however clearly be seen despite the small differences we experienced in our diets. It should be noted that forage sourcing for this trial took place during a time of extreme drought in South Africa meaning forage quality in general was very poor, even for the Hd forages. This resulted in diets with high indigestible fibre content, lower than expected dNDF, resulting in lower nutrient density and, likely, in higher filling effect. This could explain the low DMI

(16.13 to 18.47 kg/d) and its resulting decreased milk production as recorded in this research experiment in comparison to those of Alstrup *et al.* (2014) which recorded a minimum of 21.9 kg/d DMI, which is also consistent with other studies that were reviewed (Lee *et al.*, 2012; Giallongo *et al.*, 2015).

Milk quality however remained relatively constant with protein and fat concentration above the acceptable minimum for full fat milk as required by MilkSA. Milk prices however are based on yield only, without consideration being given to composition. Diet was however shown to have an effect on milk fat % (P = 0.0046) which is the qualitative feature that milk is most commonly rated according to MilkSA. However, this rating of milk quality is more relevant to Jersey herds which naturally produce much higher levels of butter fat for which a premium is received by farmers. Thus, it may be of interest to perform this experiment on breeds such as the Jerseys to determine if milk quality parameters may indeed increase income to provide a significant economic benefit to these farmers.

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Chapter 6:
Critical Evaluation

Our experiment was biased by logistics that resulted in very low differences in terms of fibre quality and protein of the specific diets. For the forages, one of the initial two oat hays that were sourced for the trial was stolen shortly before the trial was set to start. Unfortunately, due to the severe drought experienced in South Africa at the time, replacement forages were extremely difficult to source and we were forced to use what was available, even though its quality was lower than we had planned. Also, because of the low forage quality due to the drought, for both hays used, forage inclusion could not be higher than 40% to allow the desired metabolisable energy. Higher forage inclusion may have resulted in larger differences. To increase difference in fibre quality, between higher and lower digestibility treatments, wheat straw was included to the amount of 2.9% of the pellet. The presence of straw probably resulted in decreased intake for the Ld diets, resulting in significant difference for the lower CP diet (i.e. LpLd) showing how protein can counteract the lower forage quality, by stimulating fibrolytic bacteria. This is confirmed by the fact that the same diet resulted in the highest energy efficiency diet. Due to the lower than expected CP levels in the Lp diets, the Hp diets resulted in the highest milk yield and quality and therefore no conclusions can be done in terms of higher quality forage and lower dietary protein.

A repeat of this study would definitely be recommended. The use of better quality forages with the aim of improving DMI could very likely prove to have different results with regard to NUE as well as economic benefit to farmers. Due to logistical constraints, only 4 cows were available for this trial. The use of a larger sample group would also be recommended as few data points made many of the statistics inconclusive. Some data points were also lost due to illness in one of the animals and a larger sample group would reduce the effects of such occurrences.

Appendices

Appendix A

Company	Period	Cow	Diet	Day	Fat (%)	Protein (%)	Lactose (%)	SCC (x10³/ml)	MUN (mgN/dl)
LNR	1	1	A	Mon	3,58	3,13	4,66	138	15,38
LNR	1	2	B	Mon	3,35	3,02	4,68	87	21,73
LNR	1	3	C	Mon	3,91	2,82	4,93	50	14,67
LNR	1	4	D	Mon	3,21	3,00	4,58	141	21,61
LNR	1	1	A	Tue	4,15	3,17	4,57	161	14..98
LNR	1	2	B	Tue	3,47	2,96	4,69	73	20,14
LNR	1	3	C	Tue	3,34	2,76	4,93	63	13,91
LNR	1	4	D	Tue	3,57	3,05	4,64	154	20,12
LNR	1	1	A	Wed	3,90	3,16	4,61	196	13,01
LNR	1	2	B	Wed	3,78	2,98	4,65	77	20,13
LNR	1	3	C	Wed	3,65	2,81	4,95	68	13,36
LNR	1	4	D	Wed	3,50	3,00	6,47	204	20,43
LNR	1	1	A	Thu	3,59	3,14	4,72	165	10,63
LNR	1	2	B	Thu	3,78	2,97	4,67	99	16,50
LNR	1	3	C	Thu	3,40	2,85	4,84	46	12,07
LNR	1	4	D	Thu	3,57	3,01	4,53	174	17,45
LNR	2	1	B	Mon	3,23	3,13	4,73	39	16,52
LNR	2	2	C	Mon	2,60	3,27	4,60	77	17,12
LNR	2	3	D	Mon	3,28	2,90	4,83	14	20,43
LNR	2	4	A	Mon	2,25	3,26	4,43	81	20,41
LNR	2	1	B	Tue	3,08	3,14	4,65	25	16,41
LNR	2	2	C	Tue	2,70	3,33	4,64	74	16,48
LNR	2	3	D	Tue	3,21	2,95	4,88	13	16,91
LNR	2	4	A	Tue	2,15	3,27	4,61	98	15,57
LNR	2	1	B	Wed	3,05	3,21	4,53	32	15,91
LNR	2	2	C	Wed	2,53	3,42	4,70	57	15,87
LNR	2	3	D	Wed	3,22	2,95	4,83	23	16,28
LNR	2	4	A	Wed	2,19	3,29	4,65	70	13,67
LNR	2	1	B	Thu	3,30	3,29	4,54	31	18,77
LNR	2	2	C	Thu	2,91	3,36	4,69	67	16,35
LNR	2	3	D	Thu	3,28	2,98	4,84	24	17,92
LNR	2	4	A	Thu	1,85	3,28	4,62	55	13,92
LNR	3	1	D	Mon	3,03	3,36	4,53	151	24,34
LNR	3	2	A	Mon	Sour milk sample				
LNR	3	3	B	Mon	2,75	3,06	4,76	70	14,70
LNR	3	4	C	Mon	2,60	3,20	4,42	276	12,94
LNR	3	1	D	Tue	3,44	3,18	4,57	61	18,35
LNR	3	2	A	Tue	3,32	3,35	4,55	138	15,06
LNR	3	3	B	Tue	3,75	2,96	4,78	76	15,74

LNR	3	4	C	Tue	3,11	3,41	4,18	1192	14,61
LNR	3	1	D	Wed	3,39	3,21	4,51	50	19,43
LNR	3	2	A	Wed	2,78	3,30	4,63	114	16,25
LNR	3	3	B	Wed	3,57	2,87	4,85	46	15,32
LNR	3	4	C	Wed	2,95	3,24	4,40	650	15,78
LNR	3	1	D	Thu	3,40	3,29	4,47	86	19,03
LNR	3	2	A	Thu	3,17	3,31	4,57	105	14,10
LNR	3	3	B	Thu	2,31	3,02	4,66	76	16,28
LNR	3	4	C	Thu	2,91	3,25	4,45	488	16,47
LNR	4	1	C	Mon	3,96	3,55	4,49	172	14,24
LNR	4	2	D	Mon	3,53	3,37	4,51	121	21,38
LNR	4	3	A	Mon	3,11	3,02	4,90	55	13,59
LNR	4	4	B	Mon	2,68	3,24	4,35	169	18,19
LNR	4	1	C	Tue	4,20	3,39	4,44	111	12,34
LNR	4	2	D	Tue	3,51	3,32	4,50	44	19,63
LNR	4	3	A	Tue	3,37	3,00	4,84	35	12,49
LNR	4	4	B	Tue	3,57	3,24	4,41	232	17,79
LNR	4	1	C	Wed	4,65	3,72	4,25	377	12,86
LNR	4	2	D	Wed	3,60	3,29	4,26	19	20,38
LNR	4	3	A	Wed	3,43	3,00	4,73	31	11,42
LNR	4	4	B	Wed	3,40	3,18	4,38	178	14,26
LNR	4	1	C	Thu	Sick animal				
LNR	4	2	D	Thu	4,21	3,40	4,42	125	18,61
LNR	4	3	A	Thu	3,42	3,02	4,79	42	8,66
LNR	4	4	B	Thu	3,27	3,31	4,46	167	16,79
Milkolab	1	1	A	Mon	3,38	3,20	4,62	150	15,24
Milkolab	1	2	B	Mon	3,34	3,14	4,67	100	21,95
Milkolab	1	3	C	Mon	3,71	2,87	4,93	72	13,83
Milkolab	1	4	D	Mon	3,02	3,06	4,58	162	21,20
Milkolab	1	1	A	Tue	3,88	3,28	4,55	138	15,78
Milkolab	1	2	B	Tue	3,27	3,06	4,69	88	19,80
Milkolab	1	3	C	Tue	3,35	2,79	4,91	85	13,34
Milkolab	1	4	D	Tue	3,39	3,01	4,64	141	20,91
Milkolab	1	1	A	Wed	3,80	3,27	4,60	155	14,77
Milkolab	1	2	B	Wed	3,63	3,08	4,64	113	19,52
Milkolab	1	3	C	Wed	3,49	2,85	4,96	82	13,41
Milkolab	1	4	D	Wed	3,39	3,06	4,67	202	18,61
Milkolab	1	1	A	Thu	3,52	3,23	4,71	155	10,46
Milkolab	1	2	B	Thu	3,57	3,08	4,70	120	15,12
Milkolab	1	3	C	Thu	3,22	2,92	4,87	66	11,68
Milkolab	1	4	D	Thu	3,38	3,07	4,56	190	17,68
Milkolab	2	1	B	Mon	3,28	3,27	4,69	78	19,13
Milkolab	2	2	C	Mon	2,74	3,38	4,55	95	18,47
Milkolab	2	3	D	Mon	3,20	2,98	4,80	55	23,45
Milkolab	2	4	A	Mon	2,15	3,31	4,41	118	20,38
Milkolab	2	1	B	Tue	3,13	3,25	4,64	68	19,33

Milkolab	2	2	C	Tue	2,65	3,45	4,64	85	17,51
Milkolab	2	3	D	Tue	3,20	3,01	4,89	51	18,21
Milkolab	2	4	A	Tue	2,10	3,32	4,61	160	16,76
Milkolab	2	1	B	Wed	3,04	3,33	4,56	81	17,63
Milkolab	2	2	C	Wed	2,46	3,54	4,72	93	16,13
Milkolab	2	3	D	Wed	3,20	3,02	4,87	61	17,98
Milkolab	2	4	A	Wed	2,11	3,33	4,67	112	13,83
Milkolab	2	1	B	Thu	3,27	3,43	4,55	88	19,37
Milkolab	2	2	C	Thu	2,82	3,47	4,72	112	17,21
Milkolab	2	3	D	Thu	3,26	3,06	4,87	59	20,32
Milkolab	2	4	A	Thu	1,62	3,32	4,66	114	15,26
Milkolab	3	1	D	Mon	3,12	3,42	4,57	211	27,24
Milkolab	3	2	A	Mon	Sour milk sample				
Milkolab	3	3	B	Mon	2,86	3,11	4,82	74	12,21
Milkolab	3	4	C	Mon	2,66	3,21	4,47	165	13,70
Milkolab	3	1	D	Tue	3,61	3,32	4,67	102	18,71
Milkolab	3	2	A	Tue	3,39	3,45	4,61	195	14,28
Milkolab	3	3	B	Tue	3,85	2,99	4,88	103	13,76
Milkolab	3	4	C	Tue	3,14	3,42	4,26	1524	14,24
Milkolab	3	1	D	Wed	3,59	3,33	4,58	112	17,40
Milkolab	3	2	A	Wed	2,83	3,37	4,71	121	15,66
Milkolab	3	3	B	Wed	3,65	2,90	4,93	92	14,84
Milkolab	3	4	C	Wed	2,98	3,21	4,48	756	14,15
Milkolab	3	1	D	Thu	3,62	3,41	4,52	131	18,52
Milkolab	3	2	A	Thu	3,21	3,38	4,61	161	13,60
Milkolab	3	3	B	Thu	2,34	3,03	4,74	128	14,25
Milkolab	3	4	C	Thu	2,93	3,26	4,53	547	15,76
Milkolab	4	1	C	Mon	3,98	3,62	4,56	174	18,01
Milkolab	4	2	D	Mon	3,41	3,51	4,58	110	24,98
Milkolab	4	3	A	Mon	3,11	3,01	4,96	52	12,75
Milkolab	4	4	B	Mon	2,78	3,23	4,41	179	18,50
Milkolab	4	1	C	Tue	4,33	3,45	4,52	120	15,18
Milkolab	4	2	D	Tue	3,50	3,50	4,58	100	23,94
Milkolab	4	3	A	Tue	3,29	3,01	4,91	57	13,32
Milkolab	4	4	B	Tue	3,47	3,26	4,47	213	20,40
Milkolab	4	1	C	Wed	4,70	3,80	4,33	410	14,25
Milkolab	4	2	D	Wed	3,35	3,43	4,37	87	23,86
Milkolab	4	3	A	Wed	3,34	2,99	4,80	63	12,22
Milkolab	4	4	B	Wed	3,35	3,16	4,45	172	14,61
Milkolab	4	1	C	Thu	Sick animal				
Milkolab	4	2	D	Thu	4,06	3,52	4,49	137	21,56
Milkolab	4	3	A	Thu	3,61	3,03	4,83	76	8,22
Milkolab	4	4	B	Thu	3,25	3,40	4,52	196	18,68