NITROGEN USE EFFICIENCY AND SUSTAINABLE NITROGEN MANGEMENT IN HIGH PRODUCING DAIRY FARMS

A Thesis

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Master of Science

by

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ABSTRACT

The high value of milk protein, increasing feed costs, and growing concerns for the environment have made nitrogen (N) utilization a central component in balancing dairy cow rations. The objectives of this thesis were to evaluate field usable tools to predict N utilization and excretion and help develop protocols to improve N utilization on commercial farms. Specifically this included (1) an assessment of the daily variation in bulk tank milk urea nitrogen (MUN), (2) a computer-based evaluation of the Cornell Net Carbohydrate and Protein System's (CNCPS) ability to predict N excretion, and (3) a farm level evaluation of the ability of the updated CNCPS (v6.1) to develop rations with less environmental impact. In the first study, two data sets (Set A and Set B) containing daily bulk tank (BT) MUN concentrations from commercial farms were obtained from a local cooperative. Milk urea N values were analyzed by source (Set A and B) and by farm (n = 787 and 601 for Set A and B, respectively) across three months (Jan, Feb and March). Mean MUN values from Set A and B followed a normal distribution with the greatest proportion of farms (45% and 42%, respectively) falling within the 11-13 mg/dl range. The majority of variation in both data sets was explained by variability among farms. However, ~10% was attributed to the effect of month while ~20% was unexplained. The unexplained variation could be due to differences in sampling time or technique at the BT, number of milkings, total milk in the BT and/or laboratory error. Significant differences ($P<0.05$) were detected in mean MUN concentrations among months which may be due to seasonal effects. Farmers need to be aware of this variation in order for MUN to be used as an effective management tool. In the second study, CNCPS predictions of fecal N (FN), urinary N (UN), and total manure N (MN) were compared to observed data from published studies (n=32) that completed total collection N balance evaluations on lactating dairy

cows. The results showed current CNCPS FN predictions could be improved by using the equation: FN (g/day) = (((NI (g/kg organic matter) × (1 – 0.842)) + 4.3) × organic matter intake (kg/day)) × 1.20. The CNCPS calculates UN as the difference between NI and the sum of FN, scurf N and productive N. Urinary N predictions were improved by incorporating the FN prediction described above into the current CNCPS framework and accounting for N balance biases within the model. The changes to FN and UN predictions translate into an improved prediction of total manure N (Mean square prediction error = 623, coefficient of determination = 0.96, concurrent correlation coefficient = 0.97) and have been incorporated into the latest version of the CNCPS (v6.1). In the final study, the CNCPS was used to adjust the diets of two commercial herds in western NY to improve N utilization and reduce feed costs while maintaining milk production. Crude protein was reduced by approximately 1% DM, MUN was decreased by approximately 2 mg/dl and income over feed cost was improved on both farms. In addition, thirteen herds that were producing 39.3 ± 5.1 kg of milk/cow/day (mean ± SD) on low CP diets (14.3-16.5 % DM) were characterized as examples of reachable N utilization targets. This study showed that high milk and milk protein yields can be achieved on diets supplying less than 16% CP and that N use efficiency in commercial herds can be as high as 38%. This study confirms the updated CNCPS can be successfully used to develop diets with enhance N use efficiency under the constraints of a modern commercial dairy farm.

BIOGRAPHICAL SKETCH

Ryan John Higgs grew up on a farm in the small Waikato town of Ohaupo, New Zealand. He attended Ohaupo Primary School from 1989-1996, after which he attended Hamilton Boys High School (1997-2002). Throughout his time at Hamilton Boys he spent many weekends and holidays working on neighboring dairy farms. Initially, the weekend work was aimed at servicing the debt he incurred with his parents for extracurricular activities his father wouldn't include in the family budget. However, this led to a strong interest in agriculture and the dairy industry which he pursued at Massey University from 2003-2007. At Massey, Ryan completed a Bachelor of Applied Science (honors) with a major in agriculture. His bachelor's degree had a specific focus on dairy production and animal nutrition which included his first research project looking at protein utilization in lactating dairy cows. Ryan decided furthering his education would be the best strategy for achieving his long-term career goals, but wanted to experience life outside New Zealand. Given the world-class reputation of the U.S. in nutritional research he applied for a Fulbright Ministry of Research Science and Technology Scholarship to complete graduate studies in the U.S. In February of 2007 he was offered the Fulbright Scholarship and was accepted into Masters Program in the Department of Animal Science at Cornell University. He moved to Ithaca, NY in August 2007 and began his Masters.

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LIST OF ABBREVIATIONS

AA	amino acid
ATP	adenosine triphosphate
BLUP	best linear unbiased predictor
bST	bovine somatotropin
BT	bulk tank
BTMUN	bulk tank milk urea nitrogen
ºC	degrees Celsius
CCC	concurrent correlation coefficient
CNCPS	Cornell Net Carbohydrate and Protein System
CO_2	carbon dioxide
CP	crude protein
DHIA	dairy herd information association
dl	deciliter
DM	dry matter
DMI	dry matter intake
ECM	energy corrected milk
EPA	Environmental Protection Agency
Eq	equivalents
FN	fecal nitrogen
g	gram
IGF	insulin-like growth factor
IOFC	income over feed cost
IOPFC	income over purchased feed cost
kg	kilogram
lb	pound
Lys	lysine
ME	metabolizable energy
Met	methionine

MF	milk fat
mg	microgram
mM	millimolar
mmol	millimole
MN	total manure nitrogen
MP	metabolizable protein
MSE	mean square error
MSPE	mean square prediction error
MUN	milk urea nitrogen
n	number of samples
N	nitrogen
NAEMS	National Air Emissions Monitoring Study
NDF	neutral detergent fiber
NH_3	ammonia
NI	nitrogen intake
N_2O	nitrous oxide
NO_x	nitric oxide and nitrogen dioxide
NPN	non-protein nitrogen
NRCS	Natural Resources Conservation Service
P	phosphorus
P	probability
PDV	portal drained viscera
pH	potential of hydrogen
ppb	parts per billion
R^2	coefficient of determination
RDP	rumen-degradable protein
REML	restricted maximum likelihood
RMSE	root mean square error
RUP	rumen-undegradable protein
SD	standard deviation

SEM	standard error of the mean
t	tonne
Tg	teragram
TMR	total mixed ration
TP	milk true protein
UN	urinary nitrogen
USDA	United States Department of Agriculture
WHMUN	whole herd milk urea nitrogen
Yr	year

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Dairy producers in the U.S. are currently facing market and regulatory environments that are demanding production systems of increasing environmental accountability and efficiency. Improvements in the management of nutrients such as N and P are important due to their contribution to surface water eutrophication, ground water contamination (Dou et al., 1998), and emission of ammonia and nitrous oxide to the atmosphere (Fenn et al., 2003).

Balancing a ration that meets a herd's nutritional requirements will depend primarily on farm goals. A diet that maximizes milk production may be different than a diet that maximizes efficiency or profitability. To date, there has been no direct tax associated with the amount of N excreted by dairy cows in North America. In contrast, certain European countries (Belgium, Denmark, France and the Netherlands) impose a tax on farmers based on the amount of N excreted on the farm (Jones and OECD, 2004). This type of regulation could occur in the U.S. and, if it does, will shift the nutritional goals of many farms.

In order to comply with future environmental standards, robust methods that quantify N outputs from dairy farms will be required. With current technology, directly measuring urinary and fecal N on commercial farms is impractical. Mathematical models provide the opportunity to predict N excretion and emissions using indicators that are easily attained and economically evaluated in commercial laboratories. This thesis will evaluate practical ways of improving N utilization by commercial dairy cows using nutritional concepts and advanced mathematical tools.

1.2 Nitrogen emissions on dairy farms

1.2.1 Introduction

The increasing global demand for dairy products and the constant drive to improve efficiency and profitability have resulted in a rapid consolidation and expansion of the dairy industry (Van Amburgh et al., 2008). Larger, more intensive, farming systems have led to growing concerns over nutrient density, animal welfare and human safety (Van Amburgh et al., 2008). Ammonia and nitrous oxide emissions are of particular concern due to their adverse effects on both human health and the environment. Regulation is being suggested as a means to control N emissions from farms and mitigate their adverse effects (NRC, 2003).

1.2.2 Ammonia production and volatilization

Generally, only 20% to 30% of the CP fed to dairy cows is captured in the milk (Bequette et al., 1998; Broderick, 2007; Hof et al., 1997). Feed N that is not transformed into milk protein or accreted in body tissue is excreted in the feces and urine. The majority of urinary N (UN) is in the form of urea which, when mixed with urease enzymes found in soils and feces, is rapidly converted to ammonium and ammonia gas. The conversion of urea to ammonia is a three step process involving a combination of hydrolysis, dissociation, and volatilization (Burgos et al., 2007; NRC, 2003). Microbes that produce urease are abundantly present in feces and, therefore, on barn floors and any other surface frequently exposed to manure (Monteny and Erisman, 1998; Pinder et al., 2004). The rate of hydrolysis depends on the concentration of urea in the urine and the activity of microbial urease enzymes (Muck, 1982). Temperature and pH strongly influence the enzymatic activity, and under conditions often found in practice (>10ºC, pH 8.6), hydrolysis can be extremely rapid (~2 hours; Monteny and Erisman, 1998).

The amount of ammonia volatilized is regulated in part by its ionic form in solution. The ionized and unionized forms reach equilibrium with the proportion in each state depending on the temperature and the pH of the substrate (Monteny and Erisman, 1998). The ratio of ammonia to ammonium increases at higher temperatures and higher pH's (Monteny and Erisman, 1998). When pH is below 7 nearly all the ammoniacal N is in the form of ammonium, above pH 7 the proportion of ammonia increases dramatically, and above pH 11 most is in the form of ammonia (Monteny and Erisman, 1998). Urea and ammonium are non-volatile and can not be lost to the atmosphere. However, most urine on a barn floor is around pH 8.6 which means the majority of ammoniacal N is quickly transferred into ammonia (Elzing and Monteny, 1997).

Volatilization can be described as the convective mass transfer of ammonia from the aqueous phase to gas phase. The level of volatilization depends on the equilibrium of aqueous and gaseous ammonia above the barn floor (Pinder et al., 2004). Higher temperatures will shift the equilibrium towards the gas phase and, therefore, increase emissions. Air velocity through the barn is also important as it removes gaseous ammonia away from the liquid/gas boundary and enables more volatilization to the gas phase (Monteny and Erisman, 1998). Climatic and seasonal conditions, therefore, play a significant role in determining the N loss potential at any given time (Pinder et al., 2004).

1.2.3 Ammonia emissions and environmental impact

After release, ammonia gas can be re-distributed to the land as acid rain and nitrates which can have detrimental effects on natural ecosystems. Particulates are also formed with other atmospheric chemicals which adversely effect air quality and human health

(Burgos et al., 2007; Fenn et al., 2003; Powell et al., 2007). Environmental pollution of N began rapidly increasing around 1965 when the rate at which humans created new reactive N began to exceed natural terrestrial creation, and the conversion of reactive N back to N_2 by denitrification could no longer keep up (Galloway and Cowling, 2002). As a consequence, much of this new reactive N began accumulating in various environmental reservoirs such as the atmosphere, soils, and waters (Galloway and Cowling, 2002). Ammonia emitted from dairy farms and other agricultural systems contributes to this accumulation along with emissions from industrial processes, waste disposal, biomass burning, and the generation of energy, (NRC, 2003).

Globally, animal farming systems emit approximately 20 Tg N/yr as ammonia (NRC, 2003). This equates to about half the ammonia emission from terrestrial systems each year (NRC, 2003). The U.S. contributes approximately 3 Tg N/yr to this output of which approximately 50-70% comes from animal waste (NRC, 2003; Pinder et al., 2004). On release it follows a sequential cascade, first impacting atmospheric visibility and air quality, followed by soil acidity, forest productivity, terrestrial ecosystem biodiversity, stream acidity, and finally coastal productivity (Galloway and Cowling, 2002). The residence time of ammoniacal N in the atmosphere is generally in the order of days and effects are seen locally. Therefore, control strategies and regulations should be developed on a regional basis to encompass localized environmental concerns and management practices (NRC, 2003).

Dairy cows are one of the largest livestock sources of ammonia emissions due to the high concentration of N in their urine (Pinder et al., 2004; Powell et al., 2007). Many studies have shown strong links between urinary urea concentration and ammonia

emissions (Burgos et al., 2007; Elzing and Monteny, 1997; Monteny and Erisman, 1998; Monteny et al., 2002; Smits et al., 1995; Tamminga, 1992). Changing the cow's diet to improve N utilization can substantially reduce the amount of N that is lost to the atmosphere. Smits et al. (1995) reported a 39% reduction in ammonia emissions from cows fed diets low in RDP compared to cows fed high RDP diets. Similar findings were reported by Elzing and Monteny (1997). In addition, barn design and manure management can impact environmental N losses (Monteny and Erisman, 1998). Work to improve N management, both in the animal, and in manure management systems was being pursued in the Netherlands as early as the late 70's and has continued to be a relevant area of research as regulations on farmers to reduce ammonia emissions have become increasingly stringent (Sonneveld et al., 2008). Developing protocols to improve N management at the commercial level will be important in the U.S., particularly as pressure mounts to reduce the environmental footprint of the dairy industry.

1.2.4 Nitrous oxide production and global abundance

Nitrous oxide emissions form and are released via microbial nitrification and denitrification (NRC, 2003). There are three potential sources from animal production systems which include direct emissions from animals, emissions from waste management systems, and emissions from manure applied to the land, either directly by grazing animals, or by machinery (Mosier et al., 1998). A study by Amon et al. (2006) found approximately 82% of nitrous oxide emissions on dairy farms are lost from manure storage, and 18% are lost when manure is applied to fields. Direct emissions from animals are difficult to quantify but are thought to be comparatively minor (Monteny et al., 2001).

Atmospheric concentrations of nitrous oxide have steadily increased during the industrial era and are 16% larger now than in 1750 (Figure 1.2.1; IPCC, 2001). The United Nations recently released a report that clearly suggested livestock production is a threat to the global environment. This is in part because of greenhouse gas production of which nitrous oxide is a significant contributor (Steinfeld et al., 2007; Van Amburgh et al., 2008). The long residency time of nitrous oxide (~100 years) in the atmosphere means it is distributed globally where it contributes to warming of the troposphere and depletion of ozone in the stratosphere (NRC, 2003). The global warming potential of nitrous oxide is 296 times that of CO_2 making it a potent greenhouse gas (IPCC, 2001). Given the heightened concerns over global warming and climate change, regulation of such emissions are likely.

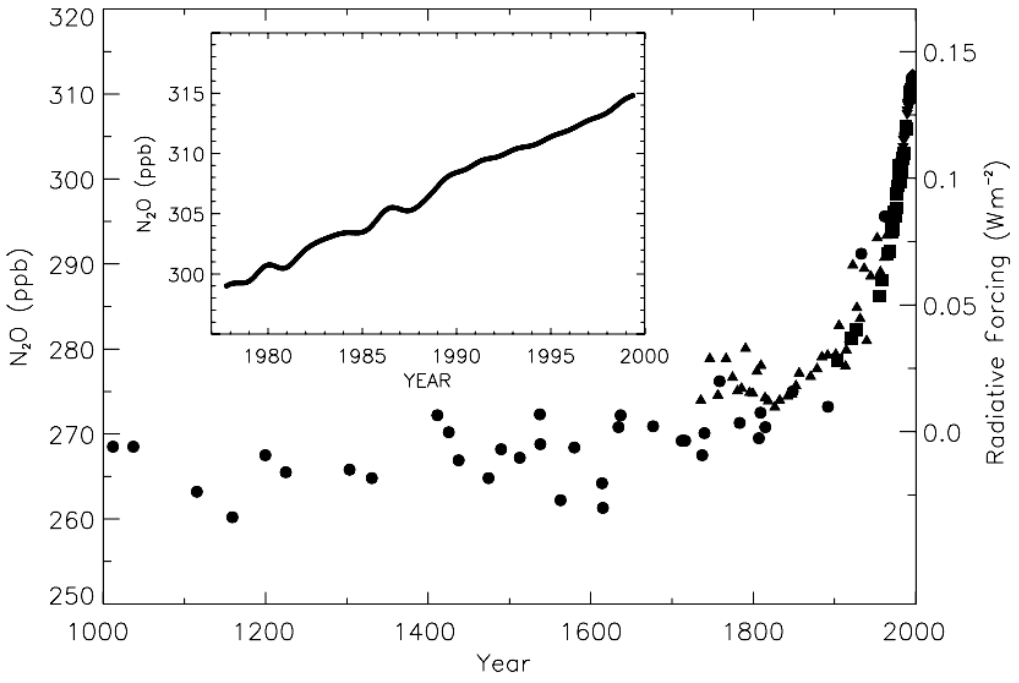

Figure 1.2.1: Change in N2O abundance for the last 1,000 years (ppb) as determined from ice cores, firn, and whole air samples. Source: (IPCC, 2001).

1.2.5 U.S. dairy production and nitrous oxide emissions

Despite the dramatic increase in global nitrous oxide levels in recent years and reports that, on a global basis, agriculture is a significant contributor (Steinfeld et al., 2007), data from EPA (2008) show that in the U.S., agriculture makes up a relatively small proportion of annual greenhouse gas emissions (8%/yr excluding sinks, and 6% including sinks; Figure 1.2.2). Furthermore, when looking at the distribution of contributing gasses, nitrous oxide accounts for only 5% of the annual emissions (Figure 1.2.3). Dairy production systems contribute 27% to annual nitrous oxide emissions in the U.S. (Figure 1.2.4), which equates to 1.0% of the total annual nitrous oxide emissions, or just 0.05% of the total greenhouse gas emissions from the U.S. each year. While reducing greenhouse gas emissions is important, these data suggest there is limited opportunity in targeting nitrous oxide from dairy production systems. Therefore, the efforts of this study will focus on ammonia, and practical ways of reducing ammonia emissions from commercial dairy operations.

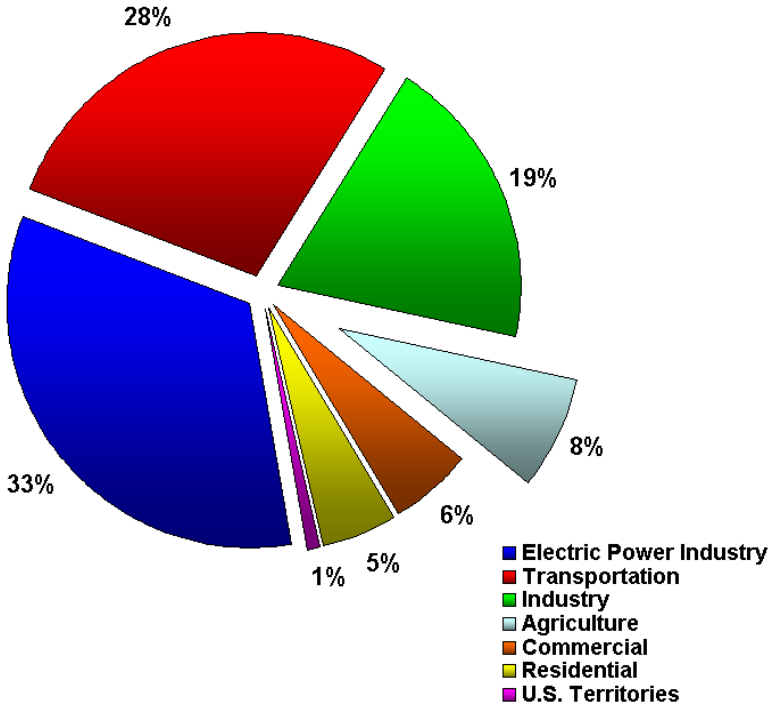

Figure 1.2.2: Greenhouse gas emission in the U.S. during 2006 allocated to economic sectors (Tg CO_2 Eq.). Adapted from: (EPA, 2008).

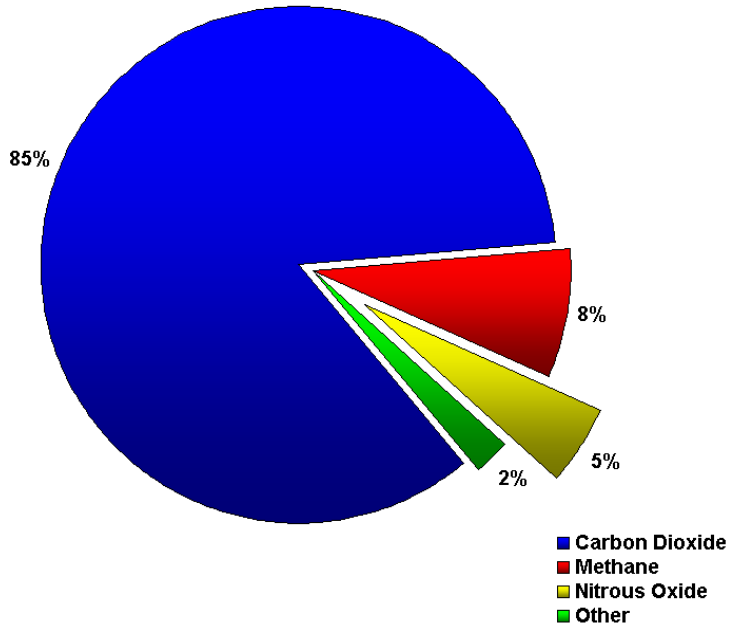

Figure 1.2.3: Greenhouse gas emissions in the U.S. during 2006 (Tg CO_2 Eq.). Adapted from: (EPA, 2008).

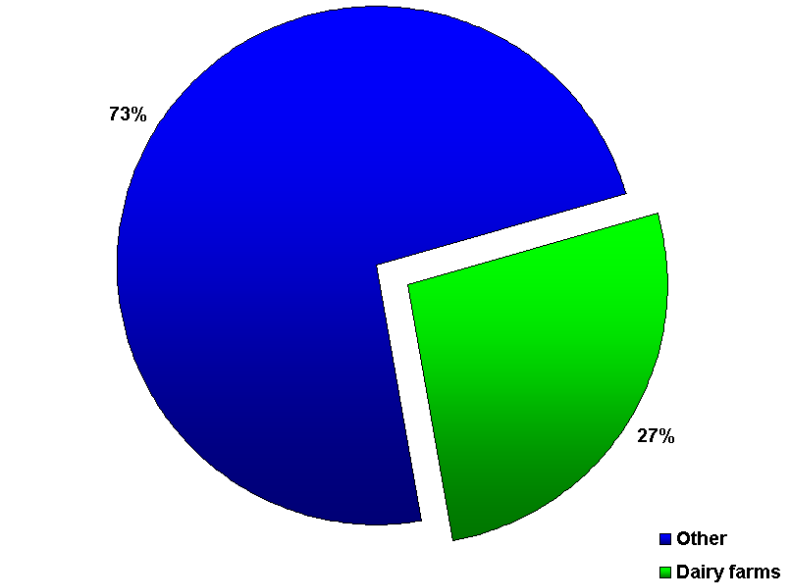

Figure 1.2.4: Contribution of dairy farms to nitrous oxide emissions from manure management in the U.S. during 2006 (Tg CO_2 Eq.). Adapted from: (EPA, 2008).

1.2.6 Current legislation and future requirements

The New York State Natural Resources Conservation Service (NY-NRCS) nutrient management conservation practice standard (NY-NRCS 590) states one of its purposes is "to protect air quality by reducing N emissions (ammonia and NO_x compounds) and the formation of atmospheric particles" (NRCS, 2007). However, the standard does not quantify limits to emissions; it merely states management practices should aim to minimize N volatilization (NRCS, 2007). The growing concerns, largely bought about as farms continue to intensify and the urban-rural interface continues to grow, may lead towards regulation as a means to control emissions and mitigate their adverse effects (NRC, 2003).

The U.S. Environmental Protection Agency (EPA) and Department of Agriculture (USDA) have recognized the need to base future regulations on sound scientific information (NRC, 2003). As a result the Board on Agriculture and Natural Resources was asked to conduct a review of relevant literature data on air emissions from animal feeding operations and evaluate a scientific basis for estimating emissions of various air pollutants (NRC, 2003). Sound scientific understanding will have to include expertise from many different disciplines, starting at the farm level with nutrition, management and engineering, to atmospheric science and even epidemiology and toxicology to consider human health effects (NRC, 2003). Regulations are only going to be accepted by the public and industry if the information they are based on is defensible. This makes developing regulations complex given the large range of geographical and climatic conditions many farms operate in. Currently, both federal agencies face the same issue: "There is no comprehensive, sound, science-based set of data on emissions from animal feeding operations" (NRC, 2003). Establishing robust data sets and developing models that encompass farm to farm variation as well as regional, seasonal and climatic variation will be crucial in establishing well grounded and accepted emissions legislation. The National Air Emissions Monitoring Study (NAEMS) has been set up to address the lack of scientific data associated with ammonia emissions (NAEMS, 2006). However, NAEMS is only collecting data on 5 dairy farms across the entire country meaning the data will have limitations. Despite this, producers should (1) be encouraged that a scientific approach is being taken in addressing this issue (2) be aware that future regulations are likely, and (3) consider how this may affect their business.

1.2.7 Conclusions

Dairy production systems contribute just 0.05% to annual greenhouse gas emissions from N_2O but are a significant producer of ammonia. The high concentration of very labile N in the urine of dairy cows leads to the rapid production of ammonia gas. The residence time of ammoniacal N in the atmosphere is in the order of days so control strategies and regulations should be developed on a regional basis to encompass localized environmental concerns and management practices. Changing the cow's diet to improve protein utilization can substantially reduce the amount of N that is lost to the atmosphere. Regulations controlling N emissions are likely as pressure mounts to reduce environmental pollution. Dairy producers should be aware of the effects this may have on their business and investigate strategies to improve N management.

1.3 Protein digestion and nitrogen metabolism

1.3.1 Introduction

Ruminants have a unique system of protein digestion and metabolism that has evolved to enable subsistence in relatively poor nutritional conditions. Dietary N sources support the requirements of both the animal, and rumen microbes. However, the extensive recycling between body, gut, and lumen pools, and interactions between the animal and microbes, make determining the net supply of protein to the small intestine complex. An understanding of the interrelationships that exist with N regulation will help facilitate the development of nutritional strategies that improve N utilization, and reduce excretion to the environment.

1.3.2 Protein digestion in ruminants

Digestion of protein in the rumen is the result of the combined process of solubilisation and degradation. Solubilisation is the process whereby protein is

released from plant cells following chewing and rumination, and is an important prerequisite for degradation (Mangan, 1982; Nugent et al., 1983). The degradation process involves a variety of micro-organisms and enzymes, which act in a largely synergistic manner (Morrison and Mackie, 1996). Proteolytic digestion in the rumen releases oligopeptides which are then broken down to dipeptides and AA. A proportion of the AA are incorporated into microbial protein, but in many situations, much of the AA N is converted to ammonia by deamination (Kolver, 1998).

The extensive development of the ruminant fore stomach and pre-gastric fermentation that occurs in the rumen alters the profile of protein reaching the small intestine, largely through the transformation of nitrogenous compounds into microbial protein (Reynolds and Kristensen, 2008). Carbohydrate fermentation is the primary fuel for this process, and when there is insufficient supply, microbes will use AA as an energy source leading to ammonia accumulation in the rumen (Reynolds and Kristensen, 2008). Ammonia that is not used by microbes as an N source for growth is absorbed through the rumen wall and converted to urea in the liver (Lapierre and Lobley, 2001). These transactions are illustrated in Figure 1.3.1 where dietary N can be followed through the digestion process to its various end products including microbial N, un-degraded dietary and endogenous N, and ammonia flowing out of the rumen in digesta.

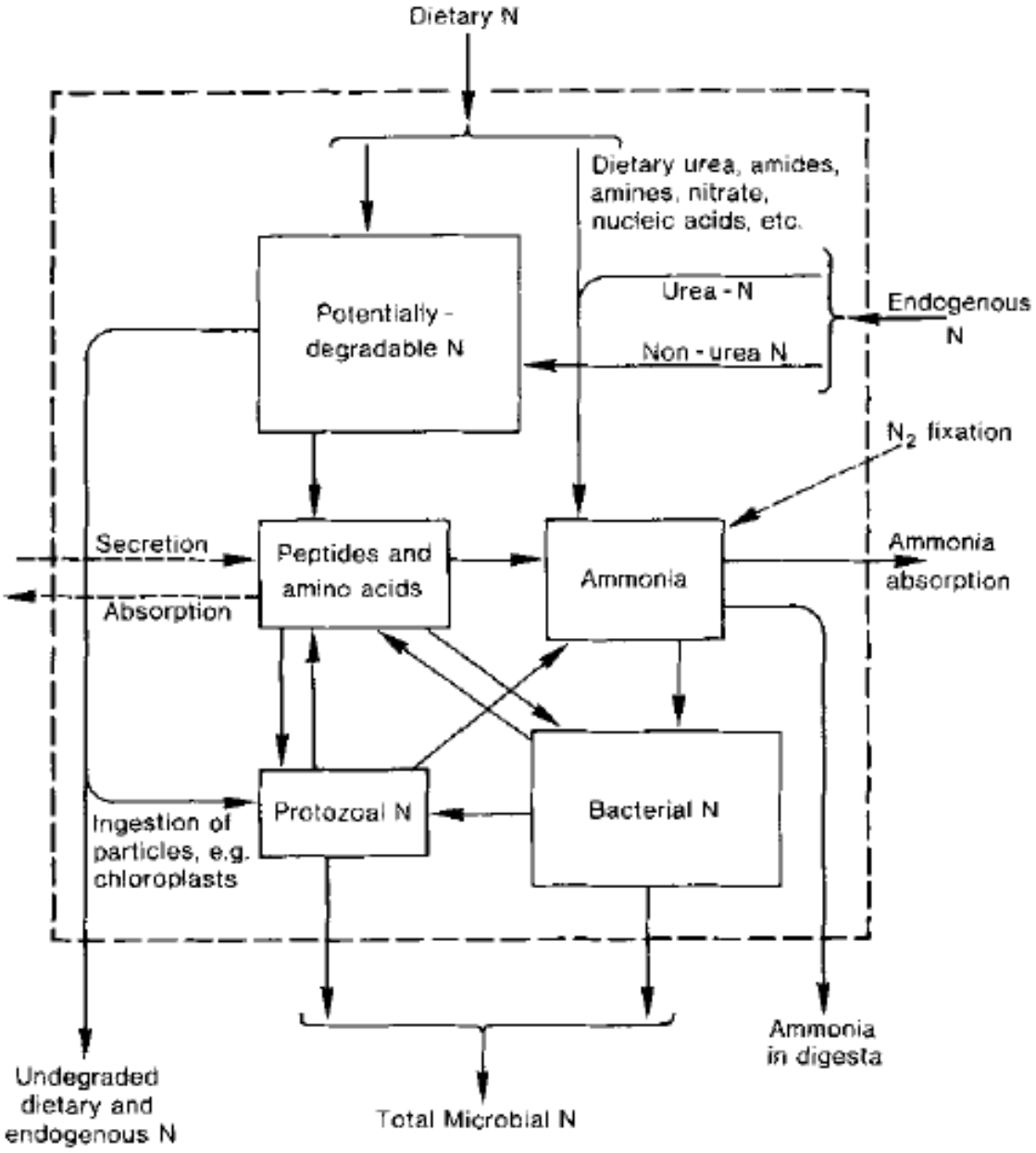

Figure 1.3.1: Graphical description of N metabolism in the rumen. Source: (Leng and Nolan, 1984).

1.3.3 Ammonia metabolism and detoxification

The symbiosis between rumen microbes and the ruminant animal, as well as the symbiosis within the microbial population itself has placed ammonia as a pivotal

component for N metabolism (Huntington and Archibeque, 2000). Ammonia is quantitatively the most important source of N for microbial protein synthesis (Kolver, 1998), and many of the cellulotic bacteria prefer, and sometimes require N in the form of ammonia (Huntington and Archibeque, 2000). This is clearly demonstrated by the fact that ruminants provided with only NPN in their diets can still sustain modest levels of production (Parker et al., 1995).

The ruminant liver plays a central role in integrating whole body N metabolism. Its positioning and vascular connection means that all tissues drained by the portal-drained viscera (PDV), including the digestive tract, must go through the liver prior to being released into peripheral circulation (Reynolds, 1992). The substantial absorption of dietary N as ammonia from the rumen requires the liver to have a large capacity to deal with this potentially toxic product (Huntington and Archibeque, 2000; Reynolds, 1992). The structure and function of the liver demonstrates the importance of this detoxification process with ornithine cycle (urea cycle) enzymes, and enzymes catalyzing transamination reactions being positioned in the mitochondria, and cytosol of periportal hepatic cells (Figure 1.3.2).

Further removal of ammonia takes place via the glutamine synthesis pathway in the perivenous parenchymal cells of the liver. These cells are located closest to the terminal hepatic venules or central veins through which blood leaves the liver enabling them to capture the ammonia that is not removed during metabolism in the periportal cells (Stipanuk, 2006). In this process glutamine is synthesized from glutamate and ammonia and plays an important role in the transfer of N between cells and tissues (Stipanuk, 2006). Glutamine released by the perivenous parenchymal cells can then circulate back to the liver and be used for ureagenesis in the periportal cells (Reynolds,

1992). This two-stage system for ammonia removal essentially eliminates all ammonia from hepatic portal blood. Other metabolic processes such as gluconeogenesis (Figure 1.3.2), acid-base regulation, and inter-organ N shuttles integrate with this system to help regulate the balance of metabolic intermediates, which, in turn, help meet the global nutrient needs of the animal (Huntington and Archibeque, 2000).

1.3.4 Nitrogen recycling

Ruminant species have the ability to survive in what would generally be considered nutritionally unfavorable conditions. Critical to this survival is the inherent ability to salvage urea N from excretion and transfer it back to the gastrointestinal tract (Van Soest, 1994). This occurs either directly, through transfer from the blood across the epithelial tissue, or indirectly through the saliva (Reynolds, 1992). Although this mechanism is not uncommon among mammalian species, in ruminants it provides a significant source of N for rumen micro-organisms which, in turn, provides the host animal with a high quality source of protein (Lapierre and Lobley, 2001).

Work which aims to quantify the sites and rates of urea metabolism in ruminants is ongoing, with recent reviews by Lapierre and Lobley (2001), and Reynolds and Kristensen (2008) providing a detailed source of current data. However, a large portion of these data were available much earlier and can be found in reviews by Huntington, (1986) and Kennedy and Milligan, (1980). Key points established by the earlier research were that urea production, excretion, and recycling are influenced by a number of factors, including dietary composition, intake, and the physiological state of the animal. Urea recycling back to the gut can be anywhere from 19-96% of endogenous production. Of this, 35-55% may be converted to further anabolic use (Harmeyer and Martens, 1980; Huntington, 1986; Lapierre and Lobley, 2001).

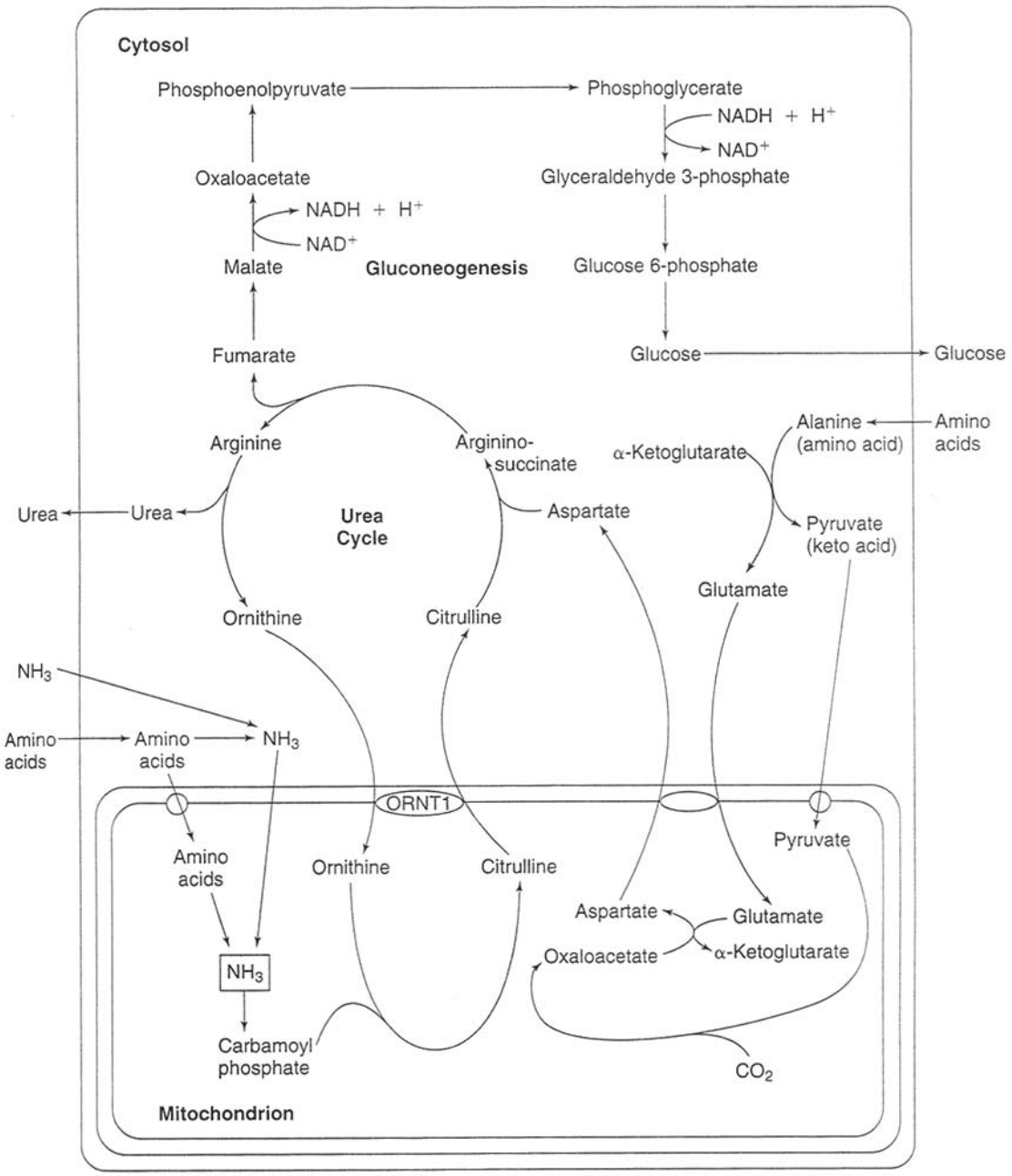

Figure 1.3.2: Metabolism of amino acids by the liver, including the partial oxidation of amino acids, gluconeogenesis, and ureagenesis. Source: (Stipanuk, 2006).

Various interactions between the animal and the diet account for the wide ranges seen in the literature, but also show the extent to which the animal can compensate for dietary N challenges (Stewart and Smith, 2005). For example, Holstein heifers have been shown to capture approximately 43% of the N recycled back to the digestive tract when fed low N diets compared to 6% when fed high N diets (Figure 1.3.3). The influx of recycled N back to the rumen has a large impact on the inflows of digestible N with 43-85% increases seen in growing steers, and 50-60% increases seen in lactating dairy cows (Lapierre and Lobley, 2001). These data suggest the role of endogenous N is significant in the ruminant, particularly when dietary N supply is low.

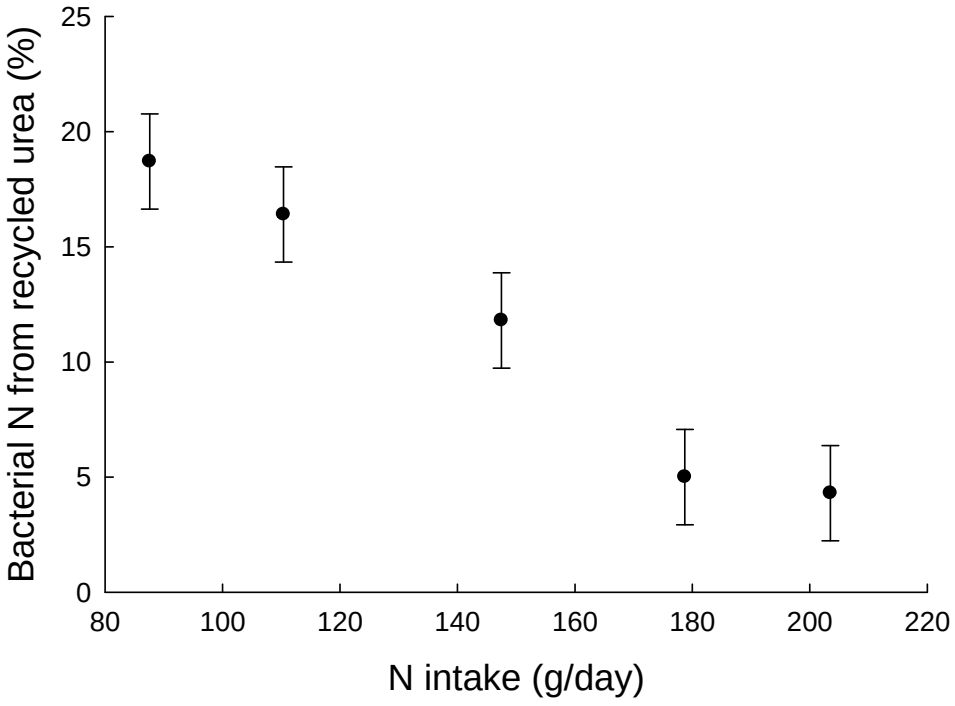

Figure 1.3.3: Proportion of bacteria flowing out of the rumen that have been synthesized using recycled N at different daily N intakes. Adapted from: (Marini and Van Amburgh, 2003).

1.3.5 Salvage mechanisms

The kidneys play an important role in salvaging urea from excretion and have specific mechanisms that modify the excretion or retention of urea depending on the metabolic needs of the animal (Harmeyer and Martens, 1980). In situations where protein supply is low, physiological changes in renal function are made that limit the drop in blood urea N and help maintain delivery of the urea to the rumen (Tebot et al., 2002). Mechanisms include a reduction in renal plasma flow, glomerular filtration rates, and an increase in urea re-absorption from the inner medullary collecting ducts (Tebot et al., 2002). Evidence of this was shown by Marini and Van Amburgh (2003) who reported no change in the amount of urea entering the gastrointestinal tract or microbial protein yield of Holstein heifers fed isocalaric diets with varying N concentrations. However, as the N concentration decreased, renal urea clearance also decreased, while gastrointestinal urea clearance and the proportion of microbial protein synthesized from recycled N both increased. In contrast, Sarraseca et al. (1998) found that as N intake decreased, urea entry into the digestive tract also decreased, both in absolute terms, and as a proportion of hepatic production, while the proportion returned to the ornithine cycle remained relatively constant. These results suggest there was no regulation in N salvaging at all. An important difference in this study compared to the study by Marini and Van Amburgh (2003) was that N intake was reduced by decreasing total DMI rather than altering the N concentration of diets with the same caloric content. Therefore, the energy to protein ratio remained constant as protein intake decreased.

In a broad sense these studies point to energy as a signal to reduce renal urea clearance and up-regulate urea recycling back to the gut. Work by Huntington (1989) and the review of Kennedy and Milligan (1980) support this suggestion with urea recycling to

the rumen shown to increase as the dietary supply of readily fermentable carbohydrates increased. In addition, Theurer et al. (2002) showed that increasing the starch availability of sorghum grain by steam flaking rather than dry rolling increased the recycling of blood urea N to the rumen and lower gastrointestinal tract of growing beef steers. This suggests mechanisms of N salvage and clearance in the ruminant seem to be more complex than a simple relationship with plasma concentration and may be regulated by energy supply.

1.3.6 Nitrogen excretion

Nitrogen consumed by lactating dairy cattle ultimately appears in either the milk, urine, feces or is interchanged with the body's N reserves (Dewhurst and Thomas, 1992). The majority of excess protein not utilized for growth or production is excreted in the urine, regardless of whether it is absorbed as AA or simply ammonia (Broderick, 2007). True endogenous N (maintenance losses) are the only real constant source of UN and account for approximately 0.35 g N/kg of metabolic weight per day (Dewhurst and Thomas, 1992). Other more variable sources include rumen degradable N not incorporated into microbial protein, metabolites of microbial nucleic acids, and products of the incomplete utilization of absorbed AA (Dewhurst and Thomas, 1992).

It is well established that the component offering the most opportunity to reduce the level of N excreted is UN (Colmenero and Broderick, 2006). A study that progressively increased the dietary N concentration of heifers from a level that was below the animal's daily requirement, to a level that was well in excess of the animal's daily requirement showed a linear increase in urea production, plasma urea, salivary urea, and urinary urea (Table 1.3.1). Nitrogen excreted in the urine was 25 times greater in the high N diet than the low N diet and urea accounted for 92% of the

additional N. These data clearly illustrate the way ruminants deal with excess N intake and show how responsive UN is to increases in dietary protein supply.

Fecal N (FN) excretion is much less flexible than UN and tends to about 0.6% of DMI (Van Soest, 1994). Evidence of this can be seen in Table 1.3.1 where as the %N in the diet increases, FN remains largely the same. Fecal N originates from a range of different sources including undigested feed N, metabolic N, endogenous N, and a small amount of ammonia (Tamminga, 1992; Van Soest, 1994). In lactating dairy cows the endogenous fraction can be relatively high due to the volume of DM going through the digestive tract and the amount of fiber in the diet (Ouellet et al., 2002). Metabolic losses, which are largely made up of microbial matter, can also be significant, particularly if hind gut fermentation is high (Dewhurst and Thomas, 1992). Despite this, there is little opportunity to reduce FN outputs in dairy cow diets apart from reducing DMI. Therefore, the manipulation of UN receives the bulk of the effort in current literature.

Table 1.3.1: Effect of dietary N intake on urinary and fecal N excretion, plasma urea N, salivary urea N, and the amount of N recycled back to the gastrointestinal tract in Holstein heifers. Adapted from: (Marini and Van Amburgh, 2003).

	N, % in the dietary DM				
	1.45	1.89	2.5	2.97	3.4
N intake (g/day)	87.6	110.4	147.5	178.7	203.5
Urinary N (g/day)	21.7	36.1	68.7	94.3	120.8
Fecal N (g/day)	46.3	49.6	49.2	52.0	50.3
Plasma urea N (mM)	1.2	3.0	6.8	10.1	13.6
Salivary urea N (mM)	1.0	2.2	5.0	7.0	10.0
Urea production (g/day)	31.1	56.1	86.9	109.8	135.2
Urinary urea N (g/day)	3.8	15.0	50.4	70.5	95.8

1.3.7 Conclusions

The extensive conversion of dietary N to microbial protein in the rumen enables ruminants to survive on diets supplying only low quality N sources. Special management is required, however, to ensure N utilization is high and N losses are minimized. In situations where protein supply is low, physiological changes in renal function are made that limit the drop in blood urea N and help maintain delivery of the urea to the rumen. Endogenous N can make up a significant proportion of dietary N intake which can subsequently be captured by rumen microbes and made available to the animal. Excess dietary N is generally excreted in the urine as urea which has important environmental consequences. Therefore, diets designed to make best use of the ruminants ability to salvage N from excretion and incorporate it into high quality microbial protein will improve N utilization and minimize environmental impact.

1.4 Feeding strategies to improve nitrogen utilization

1.4.1 Introduction

The high value of milk protein, increasing feed costs, and growing concerns for the environment has made N utilization a central component in ration balancing. Improving N utilization can be a sensitive process due to the complexities of digestion and metabolism described in section 1.3. Numerous approaches to improve N utilization have been investigated in dairy cattle including increasing ruminal energy supply, reducing dietary CP content and balancing the supply of AA to the duodenum. Endocrine signals have also been shown to have dramatic effects on milk protein output and indicate that the mammary gland functions well below its biological capacity to synthesize milk protein.

1.4.2 Dietary protein concentrations

Lactating dairy cows have specific requirements for AA that must be supplied either directly by the diet, or by rumen microbes flowing out of the rumen in the digesta (Kalscheur et al., 1999). Feeding excess CP can result in unnecessary feeding expenses with no return in milk or milk protein yield. Furthermore, the majority of excess dietary N is excreted in the urine which is the most environmentally labile form (Broderick, 2003). On the other hand, shorting the cow of AA will limit milk protein yield and revenue, which can be even more expensive than overfeeding (VandeHaar and St-Pierre, 2006). Balancing the cows metabolizable protein requirements with correct quantities of RUP and RDP, while not overfeeding, will have positive effects, not only on ration cost and profitability, but also the environment (Kalscheur et al., 1999).

Literature data have generally shown the milk yield of high producing cows (>30kg milk/cow/day) will improve as dietary CP concentrations increase (Broderick, 2003; Grings et al., 1991; Kalscheur et al., 1999; Komaragiri and Erdman, 1997; Powers et al., 1995). However, there are clearly diminishing returns and an eventual plateau where each extra unit of CP supplied is used with a lower efficiency (Metcalf et al., 2008). Broderick (2003) demonstrated this in a study that looked at the effect of feeding 15.1, 16.7 or 18.4% CP on milk protein yield over three different dietary energy levels. The study showed significant increases in milk and milk protein yield when CP was increased from 15.1 to 16.7% at the low and medium energy levels, but saw no effect of increasing CP beyond 16.7%. In addition, there was no effect from increasing CP at the highest energy level. However, milk and milk protein yield both increased as ration energy increased. Other studies by Austin et al. (1991) and Akayezu et al. (1997) reported similar protein yields in cows fed 16% CP compared to

cows fed 18% CP. Grings et al. (1991) saw responses when increasing from 13.8 to 17.5% CP but saw little benefit from increasing CP above 17.5%, and Powers et al. (1995) reported only a slight increase in milk yield and protein concentration from increasing CP from 14 to 18%. These data suggest a CP concentration somewhere between 14 to 16% DM is probably adequate to meet the requirements over a range of different dietary situations. Castillo et al. (2000) reviewed data from 91 diets fed to 580 dairy cows and concluded that CP concentrations should be reduced to 15% DM to improve N efficiency and reduce environmental impact. When compared to diets containing 20% CP this would reduce N excretion in the feces by 21% and N excretion in the urine by 66% (Castillo et al., (2000).

Sustaining high levels of production and milk protein yield on low CP diets can also be achieved using rumen protected AA. Methionine and Lys have been shown to be the most limiting AA for milk production, particularly when dietary protein supply is low (Schwab et al., 1976). Therefore, supplementing cows with additional Met and Lys in a rumen protected form can help facilitate low protein diets, and increase N efficiency (Armentano et al., 1997; Dinn et al., 1998). Responses are not always consistent, however, and can be variable especially if other key nutrients are limiting (Piepenbrink et al., 1996).

1.4.3 Carbohydrate supplementation

Rumen microbes need energy in the form of ATP to be able to capture ammonia, and use it as an N source for growth. (Nocek and Russell, 1988). Carbohydrate fermentation is the main source of ATP, and when energy supply is low, the addition of a readily fermentable carbohydrate source has been shown to enhance the capture of both dietary, and endogenous N, and increase the supply of AA to the small intestine

(Lapierre and Lobley, 2001). Kim et al., (1999) reported an increase in the microbial N output of up to 29 g/kg of carbohydrate supplemented when non-lactating cows fed grass silage had sucrose infused into the rumen. Henning et al. (1993) reported similar findings in sheep where the efficiency of microbial growth was 17% higher when a energy source was continuously infused. Studies looking at lactating cows have shown similar responses. Sairanen et al. (2005) added a cereal based supplement to cows fed fresh pasture and saw a linear increase in microbial protein available for absorption. Milk protein content was also increased and MUN decreased suggesting an improvement in total N utilization (Table 1.4.1). Broderick (2003) reported similar findings in cows fed corn and alfalfa based diets when the ratio of energy to protein was increased. However, both studies reported a drop in ruminal pH with the additional carbohydrates indicating that responses could be eroded if rumen conditions conducive to efficient fiber digestion are not maintained.

Table 1.4.1: Effect of concentrate supplementation on milk production and composition. Source: (Sairanen et al., 2005).

	Treatment[1]				***P***	
	C0	C3	C6	SEM	Linear	Quadratic
Milk yield (kg/day)						
Milk	25.1	28.3	29.6	1.42	<0.001	NS
ECM	23.6	26.8	27.6	1.56	0.002	NS
Milk composition (g/kg)						
Fat	36.8	36.6	35.5	1.74	NS	NS
Protein	31	31.5	32.7	0.5	0.03	NS
Lactose	47.4	48.3	47.9	0.56	0.06	0.008
Urea, mg/dL	50.4	47.8	38.9	2.41	<0.001	NS
Component yield (g/day)						
Fat	928	1047	1043	76.1	0.04	NS
Protein	779	889	963	47.1	<0.001	NS
Lactose	1190	1366	1417	69.3	<0.001	NS
Live weight (kg)	543	545	552	12.8	NS	NS

[1] C0 = 0 kg/d concentrate, C3 = 3 kg/d concentrate, C6 = 6 kg/d concentrate.

1.4.4 Synchronizing energy and protein supply

Synchronizing the supply of protein and energy has been suggested as another important means of improving the capture of rumen degradable protein (Castillo et al., 2000; Huntington and Archibeque, 2000). This hypothesis would mean microbes have access to high quantities of energy in synchrony with high quantities of ammonia reducing the lag in energy supply as fibrous carbohydrates are fermented (Taweel et al., 2006). Kolver et al. (1998) completed a study to test this hypothesis by feeding a non-structural carbohydrate source at the same time as high quality fresh pasture. The study measured the change in ruminal ammonia concentrations as an indicator of microbial protein synthesis. The results show the synchronized carbohydrate supplementation decreased the concentration of ammonia in the rumen by 22 to 43%, three to five hours after pasture was fed (Figure 1.4.1). This implies that, at these times, less AA catabolism occurred and ruminal N was used more efficiently (Kolver et al., 1998). However, overall the changes in the diet did not affect UN excretion or total N utilization by the cow (Kolver et al., 1998).

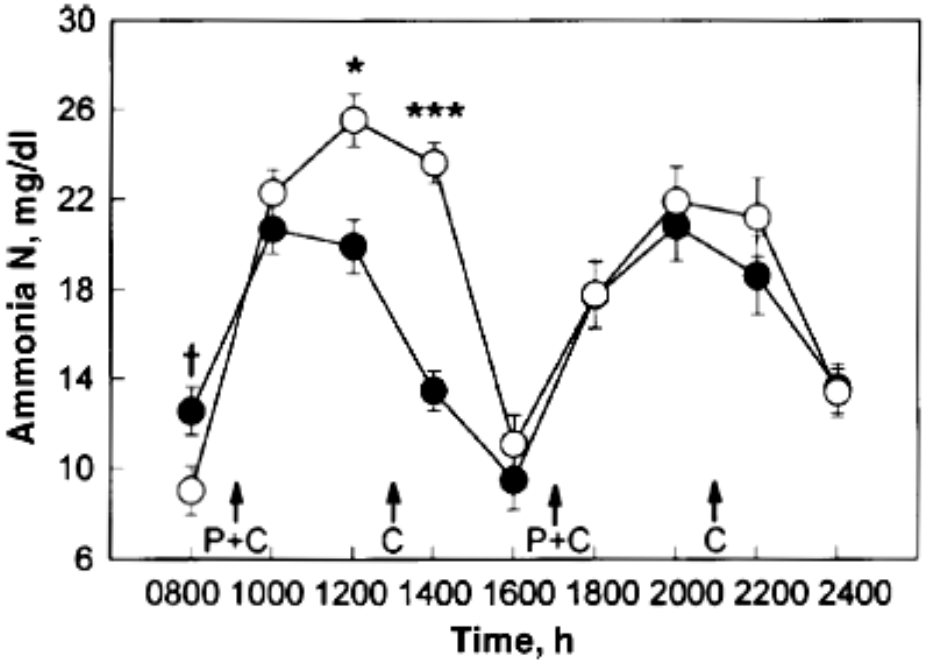

Figure 1.4.1: Diurnal pattern of ruminal ammonia N (milligrams/deciliter) of cows fed a synchronous (●) or asynchronous (○) diet. Source: (Kolver et al., 1998).

Kim et al. (1999) also tested this hypothesis in a study that infused sucrose into the rumen of cows continuously, in synchrony, or in asynchrony. The results (Table 1.4.2) suggest no advantage to synchronizing carbohydrate supply and saw the best response in microbial growth from the continuous infusion. Data from Henning et al. (1993) support these findings and suggested that dietary manipulation should be aimed at first obtaining the most even ruminal energy supply pattern, and then providing the appropriate amount of ruminally available N. This might reflect the ruminants massive capacity to recycle N absorbed from the digestive tract. Therefore, when the asynchronous diet is fed, the recycling of N via ammonia and urea enables rumen microbes to overcome any short-term effects of asynchrony making comparison between the two treatments difficult (Reynolds and Kristensen, 2008).

Table 1.4.2: The daily output of total N and purine derivatives (PD) in urine and the calculated amount of microbial N entering the small intestine in cattle receiving a basal diet of silage with or without (BASAL) intraruminal infusions of sucrose given continuously (CONT), synchronously (SYNC) or asynchronously (ASYNC). Source: (Kim et al., 1999).

	BASAL	**CONT**	**SYNC**	**ASYNC**	**SED**
Total N (g/day)	123.7	101.0	95.6	98.9	6.7[b]
PD output (mmol/day)	148.0	181.0	171.0	171.0	8.0[b]
Microbial N[a] (g/day)	84.9	113.4	104.8	104.8	6.9[b]

[a] Calculated using the equation of Susmel et al. (1994).
[b] Statistical significance of treatment effects $P<0.05$.

1.4.5 Regulation of milk protein output

Research attempting to increase the concentration and yield of milk protein has generally focused on AA supply (Mackle et al., 1999). As described above, additional dietary energy can improve ruminal ATP generation and facilitate the conversion of more ammonia into high quality microbial protein. Cows that are thought to be limited in AA supply have shown milk protein responses to additional dietary energy (Broderick, 2003; Kim et al., 1999; Moorby et al., 2006; Sairanen et al., 2005). However, in well-fed cows, responses attained by protein supplementation are often unpredictable, and considerably less than might be expected (Bequette et al., 1998; Metcalf et al., 2008). Summaries of published data have shown the relationship between milk protein percentage and dietary energy intake to be much stronger than that seen with dietary protein intake (Griinari et al., 1997). Generally, this is attributed to increased flows of microbial protein to the duodenum, but could also be explained by endocrine changes that affect the use of AA by the mammary gland (Griinari et al., 1997). Energy status affects both insulin and the IGF system which have important roles in nutrient partitioning and protein synthesis (Bauman and Currie, 1980). Interest

in this concept led to number of studies looking at the chronic effects of insulin and its impact on the regulation of milk protein synthesis (Bequette et al., 2001; Griinari et al., 1997; Mackle et al., 1999; McGuire et al., 1995).

To assess the role of insulin on milk protein regulation hyperinsulinemic-euglycemic clamps have been used to elevate circulating insulin levels (Griinari et al., 1997; Mackle et al., 1999). Dramatic increases in milk protein yield (>25%; Figure 1.4.2) were shown in well-fed cows when circulating insulin levels were elevated in combination with a post ruminal casein infusion (Griinari et al., 1997; Mackle et al., 1999). Impressive responses were also seen when insulin was infused without additional amino acids which indicates that nutrient supply *per se* probably does not limit milk production. Rather, the endocrine system places a limitation on nutrient use through metabolic regulation (Bauman, 2000; Bequette et al., 2001). On commercial dairy farms, the opportunity to improve milk protein output to the extent seen during a hyperinsulinemic-euglycemic clamp is unrealistic, given the super-physiological insulin levels required to up-regulate protein synthesis to the magnitude observed. However, the high correlation between dietary energy supply and milk protein content suggests subtle responses may be possible which are generally attributed to AA supply (Griinari et al., 1997).

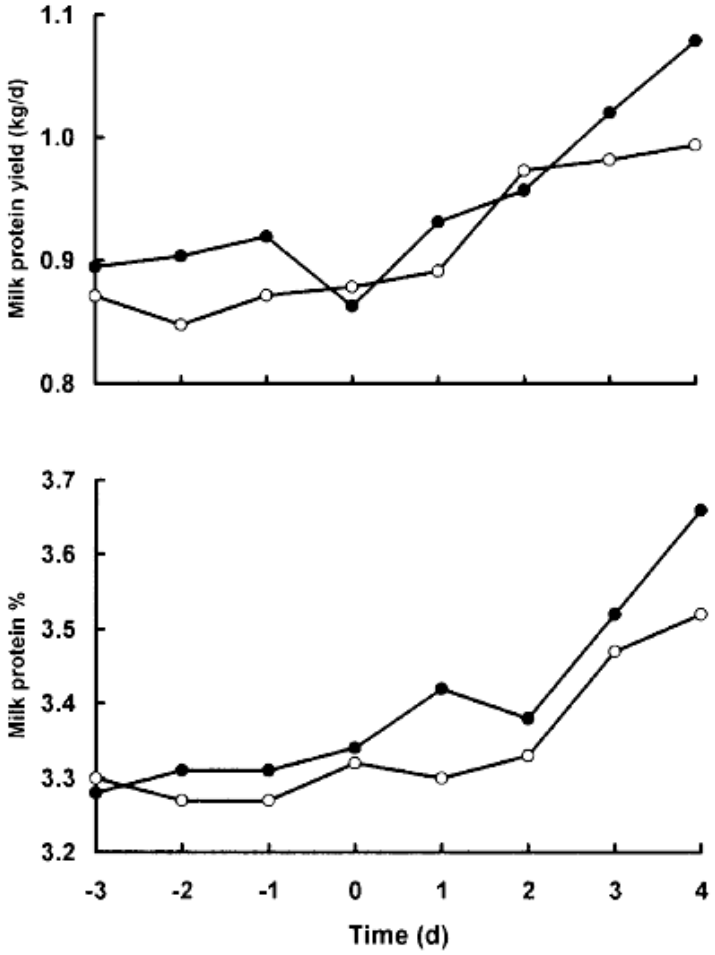

Figure 1.4.2: Effect of insulin + abomasal infusions of water (○) or insulin + abomasal infusions of casein (●) on milk protein yield, and milk protein % in lactating dairy cows during a hyperinsulinemic-euglycemic clamp. Source: (Mackle et al., 1999).

1.4.6 Implications for whole farm nitrogen balance

Improving the utilization of dietary N can translate into significant improvements in whole farm N balances and has been shown to provide the most opportunity of any part of the production system to reduce environmental N losses (Jonker et al., 2002b; Kohn et al., 1997). Figure 1.4.3 is a diagrammatic description of where N is lost at

various points in the manure management system. Nutrition can impact every level of this system through reducing the total amount of N coming out of the cow as excreta.

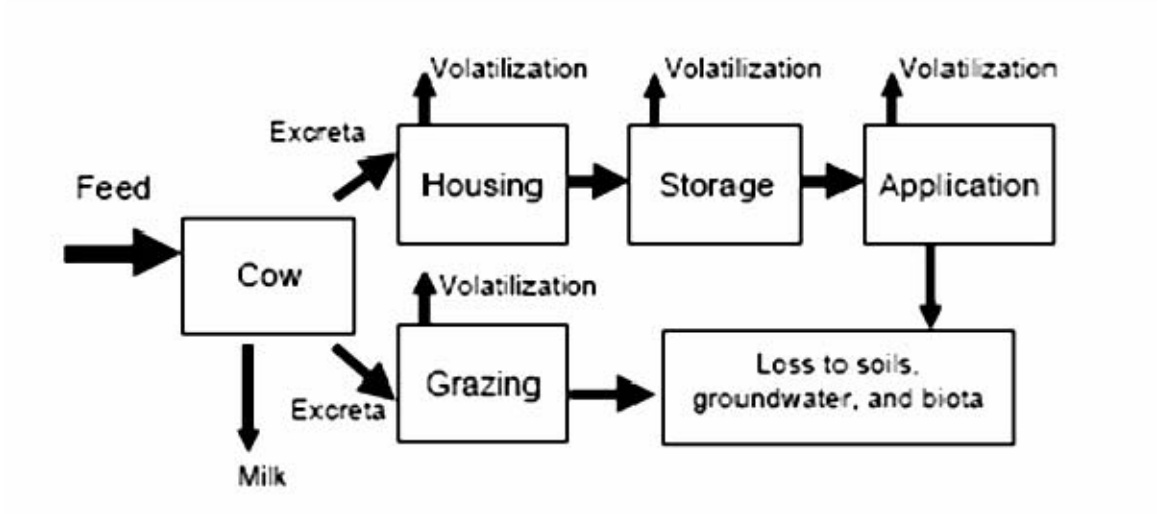

Figure 1.4.3: The flow of N into the cow and losses at various points in the manure management system. Source: (Pinder et al., 2004).

Mathematical approaches have estimated that, in dairy production systems, increasing the conversion of feed N to animal products by 50% would reduce environmental N losses by up to 40%. In contrast, reducing losses from other areas in the system, such as manure storage, collection and application by 100% would reduce total N losses by only 14% (Kohn et al., 1997). Case study farms that have implemented system wide N management plans have shown significant improvements in N efficiency by focusing on supplying the herd's protein requirements with precision and without overfeeding (Dou et al., 1998; Tylutki et al., 2004). Tylutki et al. (2004) conducted a five year study on a commercial dairy where they implemented a system wide nutrient management program and saw a 17% reduction in N excretion as well as significant improvements in whole herd production and profitability. Similar findings were shown by Dou et al. (1998) who concluded that by implementing a nutritional management

program on a herd that had no nutritional strategy, manure N could be reduced by 10%, and milk production increased by 20% (Dou et al., 1998). Whole farm implications for improving N utilization include reductions in purchased feed costs, reductions in on-farm nutrient loading and less risk of N pollution to water resources and the atmosphere (Dou et al., 1998; Kohn et al., 1997; Tylutki et al., 2004).

1.4.7 Conclusions

Balancing the cows MP requirements with correct quantities of RUP and RDP while not overfeeding will have positive effects, not only on ration cost and profitability, but also the environment. A variety of studies looking at the effects of dietary CP concentration on milk protein yield have seen little benefit from feeding over 16% CP and suggest significant reductions on fecal and urinary N can be achieved by reducing dietary CP to approximately 15% DM. Feeding adequate readily fermentable carbohydrates is critical in ensuring the microbial capture of rumen available N is efficient. Intuitively and mechanistically, synchronizing the supply of energy and protein should improve the capture of N by rumen microbes. However, evidence over a large number of studies has produced conflicting results. Synchronization has been shown to reduce ammonia concentrations in the rumen. However, these changes were transient and did not affect overall animal performance. The best improvements in N utilization have been reported when readily available carbohydrate supply is continuous, suggesting diets should be aimed at providing the most even pattern of energy supply. Dramatic increases in milk protein yield can be achieved by manipulating endocrine signals. This indicates in well-fed cows, nutrient supply *per se* does not limit milk production. Rather, the endocrine system places a limitation on nutrient use through metabolic regulation. The high correlation between dietary energy

supply and milk protein yield suggests subtle increases in milk protein can be achieved by targeting high energy rations.

1.5 Nitrogen management tools

1.5.1 Introduction

To comply with current environmental standards and anticipated air quality regulations, robust methods that quantify N outputs from dairy farms are required (Thomassen and de Boer, 2005). Directly measuring urinary and fecal N is impractical on commercial farms meaning quantification must be based on estimations rather than measured data. Mathematical models provide the opportunity to predict N outputs and emissions using indicators that are easily attained and economically evaluated in commercial laboratories. One such indicator is MUN which can be used to predict UN output (Jonker et al., 1998; Kauffman and St-Pierre, 2001; Nennich et al., 2006; Zhai et al., 2007) and also ammonia emissions (Burgos et al., 2007; Monteny et al., 2002). Other studies have used dietary and animal parameters to predict fecal N output (Huhtanen et al., 2008; Marini et al., 2008) and total manure N output (Hollmann et al., 2008; Kebreab et al., 2002; Nennich et al., 2005; Thomassen and de Boer, 2005; Yan et al., 2006).

1.5.2 Predicting nitrogen excretion using dietary indicators

Modern dairy farms generally have good records of milk composition, milk yield, ration composition and DMI. Assuming cows are at a zero N balance and are in steady state conditions, estimating manure N becomes a simple matter of accounting (Hegsted, 1976; Waterlow, 1999). Dietary N that does not appear in the milk must either be retained by the animal, or be excreted in the urine and feces (Dewhurst and Thomas, 1992). Retained N can be estimated based on live weight changes and stage

of pregnancy (NRC, 2001) meaning manure N is simply the residual. Establishing the correct partitioning of N between the feces and urine becomes more complex, and requires estimations based on dietary and animal characteristics.

Numerous studies on factors that influence manure N have been published, but most are based on individual cow data, which can confound inputs and generate biased predictions (Huhtanen et al., 2008). Meta-analytical approaches provide the opportunity to combine findings from a collection of studies and develop regression estimates that encompass more diverse situations (St-Pierre, 2001). As described in section 1.3.6, FN is much less variable than UN and closely linked to DMI suggesting FN may easier to predict. Urinary N could then simply be calculated by difference. Huhtanen et al. (2008) and Marini et al. (2008) used large data sets to derive equations with the ability to accurately predict FN (RMSE = 8.92 and r^2 = 0.98, respectively). Such equations may be relevant for practical on-farm use and provide a way to correctly estimate the partitioning of manure N to the feces and urine.

1.5.3 Using MUN as an indicator of N utilization and excretion

Regular testing of MUN offers a useful management tool to monitor the efficiency of protein utilization in the herd, while controlling feed costs, and improving milk production (Jonker et al., 1999; Kauffman and St-Pierre, 2001). Milk urea N is not a direct measure of ruminal N utilization and depends on the assumption that the fractional rate of urea clearance from the kidneys is constant (Burgos et al., 2007). However, it is a convenient measure for large-scale testing and it is strongly related to UN output (Burgos et al., 2007; Jonker et al., 1998; Kauffman and St-Pierre, 2001; Nennich et al., 2006). Urea can represent up to 90% of the N in the urine of dairy cattle and has the greatest potential for ammonia volatilization (Burgos et al., 2007).

Measuring MUN can, therefore, provide a useful method of estimating UN output and ammonia emissions without requiring detailed analyses of animal and dietary information.

Technological advances and laboratory automation of MUN analysis have seen widespread adoption of the service to producers through the Dairy Herd Information Association (DHIA) (Kauffman and St-Pierre, 2001). In addition, bulk tank MUN (BTMUN) is generally provided each time the milk is collected by the milk processor giving producers a regular update of whole-herd N status. Bulk tank MUN should theoretically be equal to the weighted average of individual MUN's from each cow in the herd if every parameter is measured without error. However, when whole herd MUN (WHMUN) is calculated and compared to BTMUN discrepancies can arise from imprecise milk weights, sampling variance, analytical variance and lab analysis (Arunvipas et al., 2004). Bulk tank MUN has additional limitations in that it is the pooled average of the entire herd so it can not be used to diagnose group-specific problems. Despite this, a study in Canada comparing BTMUN and WHMUN showed a good correlation (Concurrent correlation coefficient (CCC) = 0.91) over a wide range of diets, farming systems and varying sampling protocols (Arunvipas et al., 2004). These data suggest that, although there is some variation associated between the two measures, BTMUN can give a relatively reliable estimation of WHMUN in a range of situations. Furthermore, if the typical variation of a specific farm is known, the directionality and scale of a change in BTMUN could indicate potential problems and prompt further investigation using individual cow DHIA testing. An assessment of data encompassing management systems and laboratories in the Northeastern U.S. may give a better estimation of the variance that can be expected locally, and provide insight into how to best use local data for daily herd management.

1.5.4 The Cornell Net Carbohydrate and Protein System (CNCPS)

The CNCPS is an extensive mathematical model designed to evaluate the nutrient requirements of cattle over a wide range of environmental, dietary, management and production situations. The model relies on empirical estimations of carbohydrate and protein degradation and passage rates to predict the extent of ruminal fermentation, microbial growth, and the absorption of metabolizable energy and protein throughout the digestive tract (Fox et al., 2004). Predictions also encompass differing physiological states and body reserves meaning a diverse range of situations can be evaluated (Fox et al., 2004; Tylutki et al., 2008). The amount of information required to run a simulation in CNCPS is large which may be a constraint in some situations. However, it enables a high level of precision to be achieved and provides the opportunity to develop rations that improve animal performance and the efficiency of nutrient use (Lanzas et al., 2007).

In recent years, the CNCPS has been updated to include estimates of N and P excretion which has enabled predictions to be integrated in the development of whole farm nutrient management plans (Fox et al., 2004). The model currently predicts total MN adequately but the ratio of UN to FN is under predicted (Fox et al., 2004). Urinary N is calculated as the difference between N intake and the sum of N accretion, milk N, N retained by the conceptus, scurf N and FN, meaning a misrepresentation of any of these factors will cause a subsequent error in UN. There is some known double accounting in the prediction of FN which arises from a portion of the metabolic and ash N losses also being accounted for in the microbial N fraction (Fox et al., 2004). For CNCPS to be used effectively in nutrient management plans, more accurate partitioning of MN between UN and FN is required, particularly if ammonia predictions are to be included.

Improving the efficiency of nutrient use also requires accurate predictions of how various feed fractions behave as they flow through the digestive tract. Recent evaluations by Lanzas et al. (2007) have suggested the way the CNCPS characterizes feed proteins and their associated degradation and passage rates may cause protein to be overfed. Van Amburgh et al. (2007) provides a detailed description of recent changes that have been made to improve CNCPS predictions including a re-characterization of various pool constituents, degradation rates and, passage rate assignments. The result is a model that is more sensitive to N intake and can develop rations with less environmental impact (Van Amburgh et al., 2007). The changes represent an ongoing effort to improve the CNCPS as new data become available, and the understanding of biological mechanisms improve (Van Amburgh et al., 2007). However, field based studies are required to evaluate the efficacy of the updates to the industry.

1.5.5 Evaluating models

A model designed to predict N excretion can be validated by comparing model predictions against measured data. Published studies that have completed total collection N balance assessments provide a source of data that has been measured in a controlled situation. However, consideration of some of the inherent error associated with balance studies is important. Failure to do so may result in unfair conclusions regarding a models predictive ability and lead to unnecessary adjustments, ultimately hindering the models development. One of the major issues with N balance studies is the overestimation of retained N that can arise from losses incurred during collection and analysis (Spanghero and Kowalski, 1997). Bockmann et al. (1996) completed a study that assessed how well N balance studies accounted for N intake and found that, on average, 6% of the N intake could not be recovered in the milk, feces or urine.

Studies that have attempted to quantified losses from balance studies have been unable to identify any large losses that are not accounted for in standard measurements but highlight that multiple minor losses, in total, may be important (Reynolds and Kristensen, 2008). However, a study by Juko et al. (1961) showed that approximately 12% of CP is lost from fecal material when drying before analysis and warrants careful consideration.

Meta-analysis approaches offer a powerful way to compare large data sets from many different studies without the inherent bias and large type II error that results from using unbalanced predictor variables (St-Pierre, 2001; Tedeschi, 2006). Historically, the effect of study had to be included as a fixed effect in regression models because of the inability of statistical software to efficiently solve even modest sized mixed models (St-Pierre, 2001). Modern software has overcome this limitation and now the effect of study, and its interaction, can be included in a mixed model as random components, giving stronger prediction equations, and a better indication of the source of error (St-Pierre, 2001). Despite this, the shortcomings in N accounting that arise from the inadequacies in N balance studies must still be considered as they may help explain why a model appears to be over-predicting manure N, and incorrectly partitioning N between the feces or urine.

1.5.6 Conclusions

Mathematical models provide an advanced method of strategically improving N utilization and animal performance using inputs that are easy to collect, and economically measured. Models such as the CNCPS are continually being updated and improved as new data become available and the understanding of biological mechanisms improves. The evaluation process is an essential step in model

development as it indicates the level of accuracy and precision of model predictions (Tedeschi, 2006). It also helps validate whether the model accomplishes what it is designed for and provides evidence that will augment the confidence of it users.

1.6 Conclusions and Objectives

Dairy cows are one of the largest sources of N emissions from livestock in the U.S. The high concentration of very labile N in the urine leads to the rapid production of ammonia gas. Regulations controlling N emissions are likely to occur as pressure mounts to reduce the environmental footprint of food production. Dairy producers should be aware of how regulations may affect their business, and investigate strategies to improve N management.

The extensive conversion of dietary N to microbial protein in the rumen provides both opportunities and challenges for dairy production systems. In situations where protein supply is low, physiological changes in renal function are made that salvage urea from excretion and help maintain delivery of the urea to the rumen. When dietary protein is low, recycled urea can make up a significant proportion of N flowing into the rumen which can subsequently be captured by rumen microbes and made available to the animal. However, excess dietary N is generally excreted in the urine as urea which can have important environmental consequences. Balancing the cows MP requirements with correct quantities of RUP and RDP while not overfeeding will have positive effects, not only on ration cost and profitability, but also on the environment. Literature data have shown little benefit from feeding over 16% CP in the diet and suggest significant reductions on fecal and urinary N can be achieved by reducing dietary CP to approximately 15% DM. The addition of a readily fermentable

carbohydrate source has been also been shown to enhance the capture of both dietary and endogenous N and can help drive milk protein output.

Mathematical models provide an advanced method of strategically improving N utilization and animal performance using inputs that are easy to collect, and economically measured. One such indicator is MUN which can be used to predict UN output and also ammonia emissions. More extensive models such as the CNCPS have been designed to precisely evaluate the nutrient requirements and excretion of cattle over a wide range of environmental, dietary, management and production situations. The evaluation process is an essential step in model development as it helps validate whether the model accomplishes what it is designed for and provides evidence that will augment the confidence of it users.

The objectives of this thesis are to evaluate field useable tools to predict N utilization and excretion on dairy farms, and help develop practical protocols to reduce N excretion to the environment in the commercial situation. This will include three separate studies:

1. A statistical assessment of the daily variation in bulk tank MUN data from farms in the Northeastern U.S. The objectives are to get a better estimation of the variance that can be expected locally, and to provide insight into how to best use local data for daily herd management.

2. A computer-based study evaluating the ability of the CNCPS v6.1 to predict fecal, urinary, and total manure N excretion. The objectives are to assess the

adequacy of the current equations and to facilitate the development of alternative equations if necessary.

3. A farm level study using CNCPS v6.1 as a tool to reduce N excretion on commercial dairy farms while maintaining high levels of milk production and the economy of the ration. Farms that are successfully producing high milk yields on low protein rations will be monitored as examples of reachable targets. The objectives are to develop management protocols to successfully reduce N outputs on commercial dairies, to document the N use efficiency of farms that are industry leaders, and to validate the efficacy of the updated biology in CNCPS v6.1.

CHAPTER 2: VARIABILITY IN THE ANALYSIS OF DAILY BULK TANK MILK UREA NITROGEN, MILK FAT AND MILK PROTEIN

2.1 Abstract

Milk urea N is a useful indicator of N utilization in lactating dairy cows. Recently, concerns have been raised regarding the level of variation in MUN from bulk tank (BT) samples collected during milk pick up. The objectives of this study were to characterize the level and source of variation from the BT of individual farms over one month. Milk urea N values were analyzed by source (Set A and B) and by farm (n = 787 and 601 for Set A and B, respectively) across month (Jan, Feb and March). Both data sets were analyzed in the same laboratory using the same analytical equipment. Mean MUN values from Set A and B followed a normal distribution with the greatest proportion of farms (45% and 42%, respectively) showing MUN values within the 11-13 mg/dl range. Data from Set A had slightly less variation in MUN readings than Set B. Both data sets had approximately the same number BT with a SD greater than 2 mg/dl while Set A had a greater proportion of BT with SD in the range of 0.5-0.9 mg/dl. The majority of variation in both data sets was explained by variability between farms. However, ~10% was attributed to month and ~20% was unexplained. Significant differences ($P<0.05$) were detected in mean MUN concentrations across months. Differences may also be related to herd size or level of milk production but these data were not available for the current analysis. Given the nature of the data, it is difficult to distinguish the exact source of the variation, and with only three months of data it is hard to draw conclusions. Repeating the analysis with the inclusion of herd size, milk production and more months of data would help further characterize the level of variation that may be expected in difference farming systems, and at different times of the year.

2.2 Introduction

Regular testing of MUN offers a useful management tool for farmers and nutritionists to monitor the efficiency of protein utilization in the herd, while controlling feed costs, and improving milk production (Jonker et al., 1999; Kauffman and St-Pierre, 2001). Recently there has been concern about the variability in MUN concentrations from bulk tank samples collected during milk pick up. In order for MUN to be used as an effective management tool, normal day to day variation needs to be characterized, and off farm variation needs to be minimized. This will ensure producers and nutritionists are confident that the changes they see in MUN concentrations are due to on-farm factors rather than external factors out of their control (such as laboratory error or sampling error). Producers also need to know how to react to daily on-farm variation and be able to distinguish between changes that may be due to the environment versus changes that could be attributed to the diet. The following analysis looks at the monthly variation in individual bulk tank MUN concentrations.

2.3 Materials and Methods

2.3.1 Dataset description

Two data sets (Set A and Set B) were obtained from a local cooperative. Each included three months of data (Jan-Mar 08) from individual farm bulk tank (BT) milk samples which were analyzed for MUN, fat and protein. Samples from both data sets were analyzed at the same laboratory using either a Foss 4000 or Foss 6000 system (Foss Inc., Eden Prairie, MN). It was not possible to establish exactly which machine was used on each specific sample. However, both data sets covered the same period of time and the proportion of samples analyzed by each machine, between data sets, was the same. When the mean and SD were assessed, only BT with ten or greater monthly samplings were included in the analysis; less than ten data points was considered

insufficient to generate useful statistics. All farms provided by Set A had only one BT. A small number of farms in the Set B data set had more than one BT. In this instance, the second BT was removed to ensure individual farms did not bias results. The number of times BT were sampled each month by Set A and B was also assessed. For this, each BT was categorized as having less than 10, 10-15, 16-20, or greater than 20 samplings per month. Figure 2.3.1 shows the majority of BT (70-80%) are measured 10-15 times per month, close to 20% of BT are sampled less than 10 times per month, and a small proportion are sampled more than 15 times. The size of each data set used for each analysis is in Table 2.3.1.

Table 2.3.1: Size of the data sets used to generate descriptive statistics (DS), complete a variance component model (VCM) and asses the mean and SD of MUN, milk fat and milk protein.

	DS and VCM		**Mean and SD**[a]	
	Set A	Set B	Set A	Set B
Farms	849	664	787	601
Samples	31462	24169	29135	21793

[a] The number of farms and samples used to assess the mean and SD in each data set differs compared to bulk tank readings per month due to bulk tanks with less than 10 readings per month being excluded from the analysis.

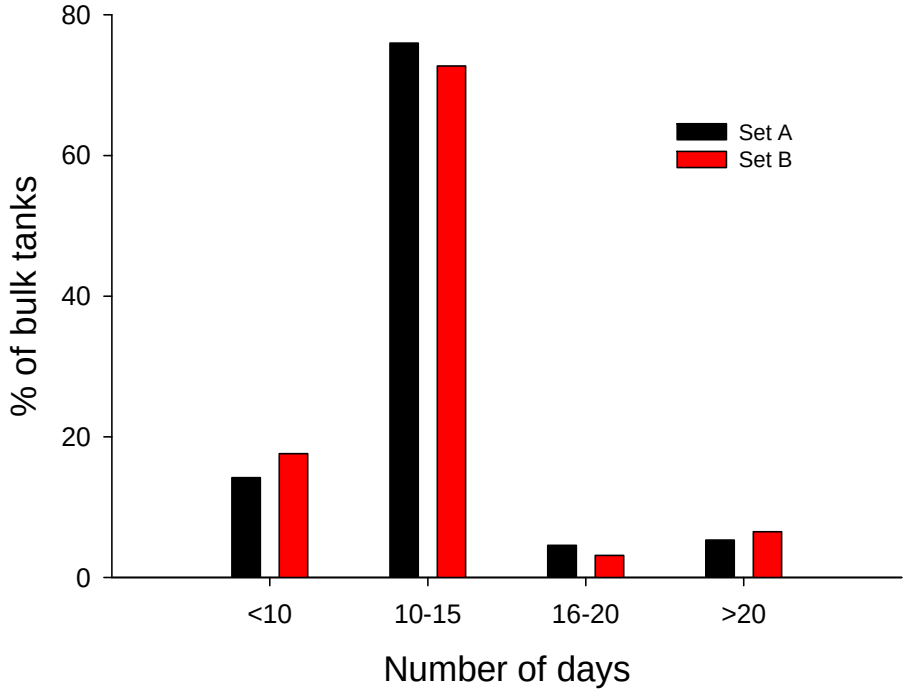

Figure 2.3.1: Number of times each month Set A and Set B reported individual bulk tanks MUN, fat and protein concentrations.

2.3.2 Statistical analysis

A basic statistical analysis was completed to assess the mean and SD associated with each milk component from individual BT samples over one month. Given that three months of data were available, most BT were represented three times in the analysis. Further assessment of the variation associated with MUN was completed with a variance component model using the REML procedure of JMP (2007). This enabled the total variation to be partitioned into that associated with the farm, the interaction between the farm and the month of sampling, and the residual. Month of sampling was also included in the model as a fixed effect and a standard least squares comparison was completed to test for differences across each of the three months (Jan, Feb and

March). The Tukey's HSD procedure was used to indicate significant differences ($P<0.05$).

2.4 Results and discussion

Descriptive statistics for MUN, fat and protein are in Table 2.4.1 for each lab. The min and max values for MUN, fat and protein show the range in each data set. Both Set A and B have a SD of close to 3 for MUN suggesting a lot of variability. However, this may be expected given the wide variety in farms and farming systems potentially represented.

Table 2.4.1: Descriptive statistics for bulk tank MUN, fat and protein concentrations measured by Sets A and B.

	Fat		**Protein**		**MUN**	
	Set A	Set B	Set A	Set B	Set A	Set B
Mean	3.87	3.82	3.10	3.07	11.77	12.64
SD	0.37	0.32	0.19	0.17	2.96	2.93
Min	1.85	1.93	2.09	1.80	1.20	1.30
Max	9.69	9.83	4.23	4.19	29.70	28.40

2.4.1 Milk urea nitrogen

Mean MUN values followed a normal distribution for both Set A and B. The majority of farms (~45%) fell within the 11-13 mg/dl range with the balance being evenly distributed either side of this (Figure 2.4.1). Approximately 5% of BT had mean MUN concentrations of less than 7 with some individual values being as low as 1.2 (Table 2.4.1). Biologically this would mean the herd was extremely deficient in both total N and RDP and have a very negative N balance. Interestingly, MUN measured in these low ranges were consistent among months (~15 samples), and also tended to be low for all three months (data not shown). This essentially rules out laboratory error and

suggests some herds are running very negative N balances. An investigation into the management system that causes these herds to be so low in dietary CP would be beneficial and could possibly lead to an intervention strategy to improve herd performance.

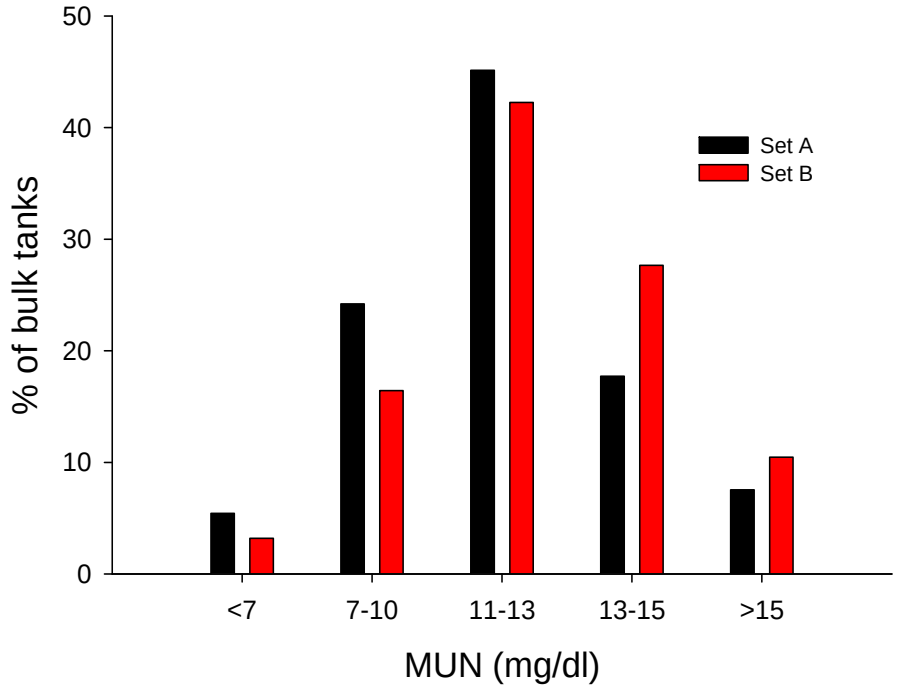

Figure 2.4.1: Mean MUN concentrations from bulk tank milk samples measured by Sets A and B.

Milk urea N SD is summarized in Figure 2.4.2 and represents the variation on specific BT for the duration of one month. Therefore, each BT is generally represented three times (Jan, Feb and March). The results show the large majority of BT varied 0.5-1.4 units from the mean. The values from Set A appeared to have less variation than from Set B, with a greater proportion of BT having a SD of 0.5-0.9 than the higher ranges. Both data sets had approximately the same number of BT with SD greater than 2

(~7%). These data show there is far less variation associated with MUN analysis when represented on a within-farm, and within-month basis, rather than a whole data set basis as described in Table 2.4.1. Care needs to be taken when interpreting data presented in this form as the mean associated with SD is not presented. However, it does indicate that the variability in some BT is very high which would make it difficult for farmers or nutritionists to use MUN as an accurate management tool.

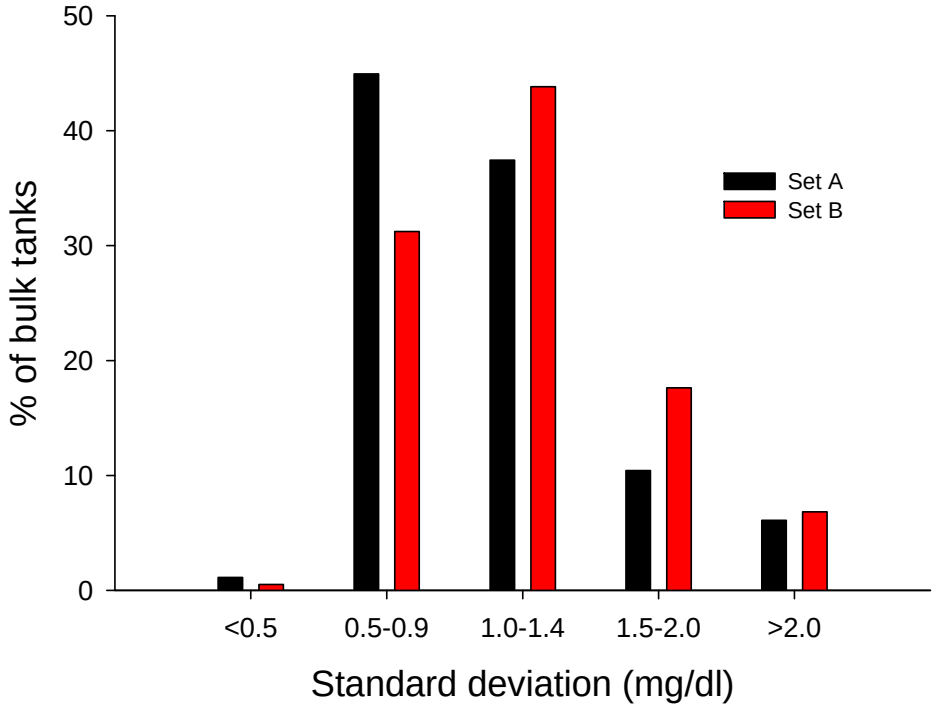

Figure 2.4.2: Standard deviation in MUN concentration (mg/dl) from bulk tank milk samples measured by Sets A and B.

More advanced statistical techniques can give further insight into the contribution of particular sources of variation within each data set. In the current situation, the variation attributed to farms and the interaction between farm and month of sampling

can be accounted for by the statistical model. Consequently, the unaccounted variation, or variation that may be attributed to off farm factors, is largely pooled into the residual. Table 2.4.2 shows farm to farm variability accounts for approximately 70% of the variation for both Set A and Set B. This is to be expected given the range in production systems represented in each data set. Interestingly, the interaction between farm and month of sampling accounted for 11% of the variation for both Set A and B (Table 2.4.2). Significant differences in mean MUN concentrations were also detected across months in both data sets (Table 2.4.3).

The difference in mean MUN concentrations from Jan to March in Set A exceeded 1 mg/dl, which given the large number of observations (Table 2.3.1), would have translated into a considerable reduction in N excreted to the environment (Burgos et al., 2007; Jonker et al., 1998; Kauffman and St-Pierre, 2001). The inconsistent trends seen between data sets and limited number of months available for analysis make it difficult to draw more detailed conclusions. However, seasonal effects of MUN have been previously documented (Arunvipas et al., 2004; Verdi et al., 1987). For example, Verdi et al. (1987) reported clear seasonal trends in the NPN concentration of milk from lactating dairy cows; NPN were highest in the summer months, and lowest in the winter months. In addition, heat stress is know to cause significant amino acid catabolism in the skeletal muscle (Fuquay, 1981). Urea equilibrates in body water so if AA acid catabolism is high, BUN and MUN will also be high (DePeters and Ferguson, 1992). Herds grazing pasture at certain times of the year have also shown significant seasonal differences (Arunvipas et al., 2004). Producers should be aware that seasonal variation does occur and MUN may be elevated during the summer, particularly during heat stress events. The current data suggest sampling time is an important

source of variation. However, causes for this are only speculative given the limited amount of information provided in this data set.

The residual variation for Set A and Set B was 17.8% and 21.1%, respectively (Table 2.4.2). This probably encompasses the variation associated with more random factors and may include:

- Variation in sampling technique at the bulk tank.
- Differences in the time the bulk tank sample was obtained each day.
- Number of milkings or total milk in the tank.
- Laboratory error.

Significant differences have been shown in the analysis of MUN between different laboratories, and different analytical methods (Peterson et al., 2004). Analytical method was shown to be of particular importance with recovery of MUN among 5 different methods ranging from 47.1% to 95.4% and standard error within method ranging from 2.8% to 10.1% (Peterson et al., 2004). Given that both data sets in the current analysis were analyzed in the same laboratory, using the same piece of equipment, laboratory error between data sets was assumed to be similar. Variation could also be due to any combination of the factors listed above and may also be related to herd size or level of milk production. These data were not available for the current analysis. Completing this analysis with the inclusion of herd size and milk production may be useful.

Table 2.4.2: Estimations of the variance components for MUN as it is related to farm, and the interaction between farm and month for each data set (Set A and Set B).

Random effect	**Variance Component**		**Percent of Total**	
	Set A	Set B	Set A	Set B
Farm	6.27	6.08	71.32	67.91
Farm × Month	0.96	0.99	10.86	11.01
Residual	1.57	1.89	17.82	21.07

Table 2.4.3: Least squared (LS) means and standard error's associated with MUN concentrations measured by Set A and Set B in January, February and March of 2008.

Month	**LS Means**[1]		**Standard Error**	
	Set A	Set B	Set A	Set B
January 2008	12.18[a]	12.73[a]	0.09	0.10
February 2008	11.80[b]	12.28[b]	0.09	0.10
March 2008	11.14[c]	12.59[a]	0.09	0.10

[1] Levels not connected by same letter within a column are significantly different ($P<0.05$).

2.4.2 Milk protein

Mean bulk tank protein concentration and monthly variation are shown in Figures 2.4.3 and 2.4.4, respectively. The range in mean concentration is what would be expected given the large number of farming systems represented and both sets have similar values. Standard deviation is generally between 0.02 and 0.05 % true protein (TP) with some BT greater than 0.07 %TP.

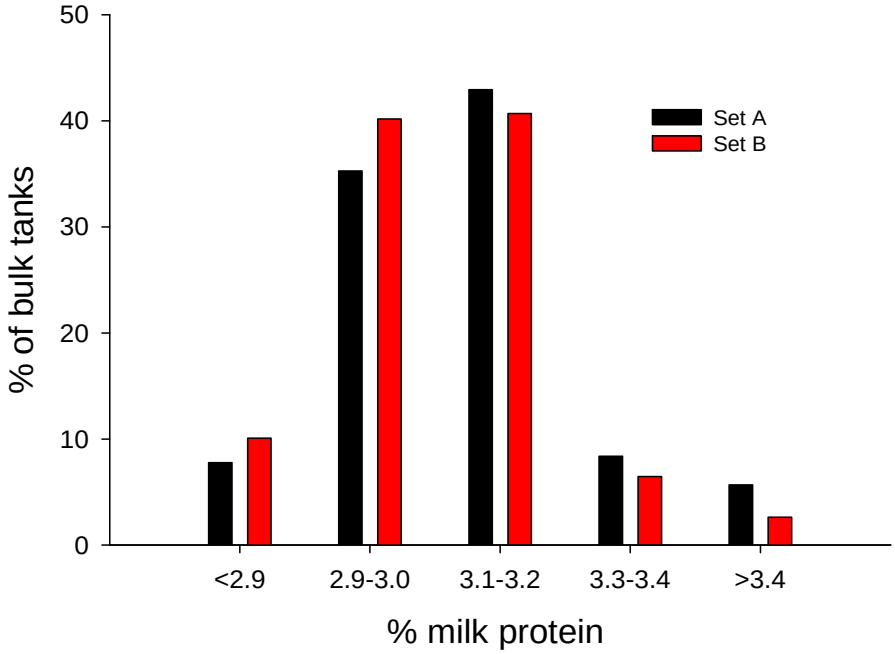

Figure 2.4.3: Mean milk true protein concentrations from bulk tank milk samples measured by Sets A and B.

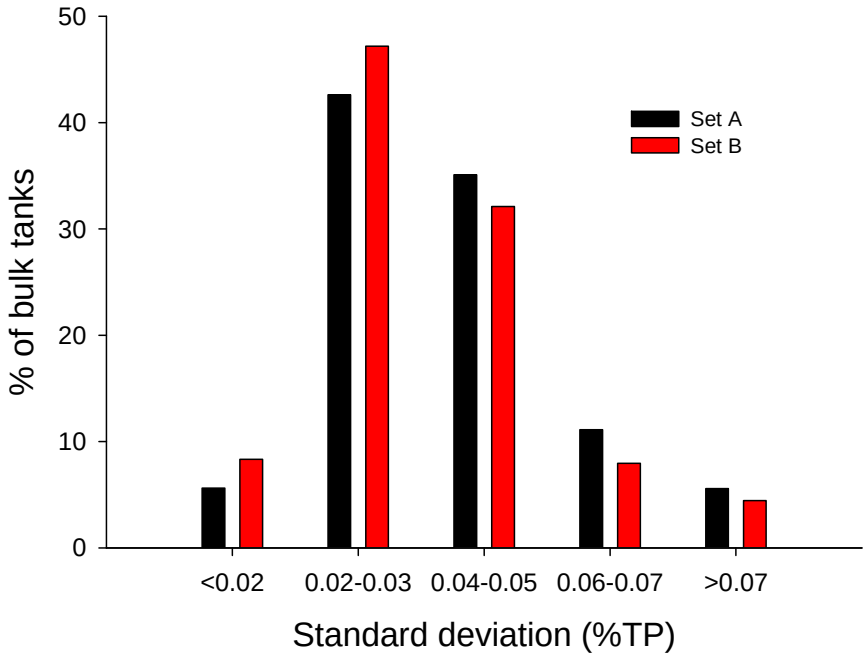

Figure 2.4.4: Standard deviation in milk true protein (TP) concentration from bulk tank milk samples measured by Sets A and B.

2.4.3 Milk fat

Mean bulk tank fat concentration and monthly variation are shown in Figures 2.4.5 and 2.4.6, respectively. The range in mean concentration is what would be expected given the size of the data set and number of different farming systems represented. Both sets have similar values. Standard deviations were generally between 0.04 and 0.11 % milk fat (MF) with some BT greater than 0.15 % MF. Variation in fat seems to be higher than protein. However, the range in the data is also higher so more variability may be expected.

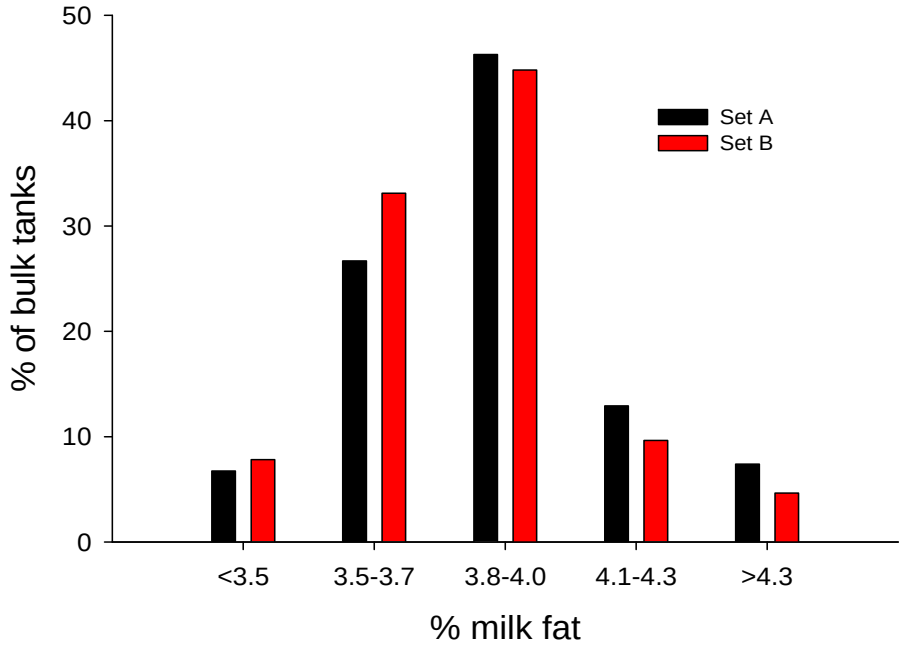

Figure 2.4.5: Mean milk fat concentrations from bulk tank milk samples measured by Sets A and B.

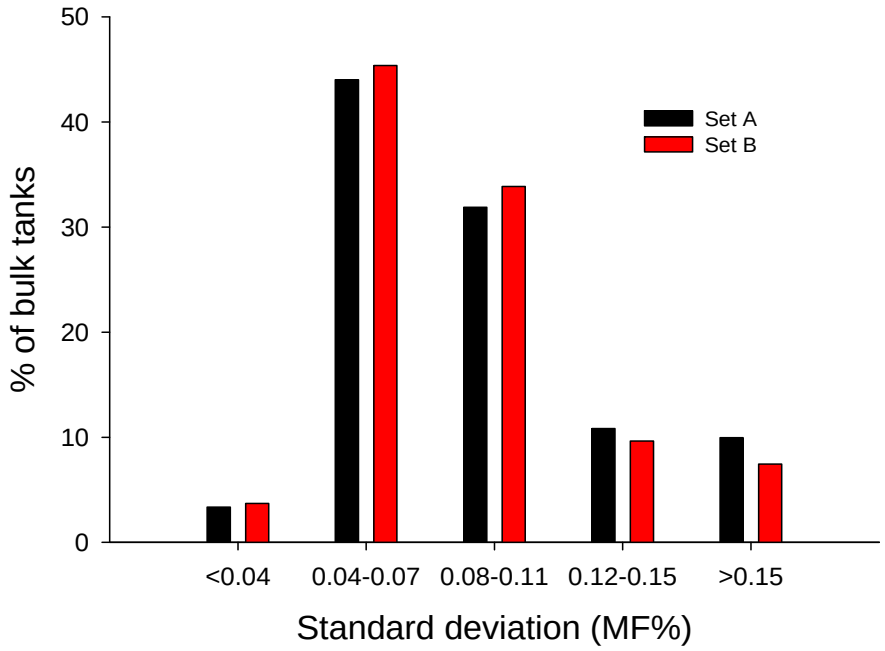

Figure 2.4.6: Standard deviation in milk fat (MF) concentration from bulk tank milk samples measured by Sets A and B.

2.5 Conclusions

There was a large range in variation for BTMUN concentration across the data sets but both Set A and Set B's data followed the same general trend. Some BT had a SD of less than 0.5 mg/dl and some had a SD greater than 2 mg/dl. However, over half the BT in each data set had a SD greater than 1 mg/dl. Set B's data appeared to have slightly more variation which could be due to a range of reasons including simple differences in the farms sampled. The variation in both protein and fat concentration is small. The variation in fat is slightly higher than protein. However, the range in the data is also larger so this may be expected.

Farmers and nutritionists need to be aware of the level of variation that's occurring on each specific farm to enable MUN concentration to be used as an effective

management tool. The source of variation also needs to be identified and minimized where possible. Differences may be related to herd size or level of milk production. These data were not available for the current analysis. Therefore, conducting a similar study with the inclusion of herd size, milk production and more months of data may be useful.

CHAPTER 3: EVALUATION OF THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM'S ABILITY TO PREDICT NITROGEN EXCRETION IN LACTATING DAIRY COWS

3.1 Abstract

Nitrogen excretion is of particular concern on dairy farms, not only because of its effects on water quality, but also because of the subsequent release of gases such as ammonia and nitrous oxide to the atmosphere. To manage N excretion, accurate estimates of urinary N (UN) and fecal N (FN) are needed. On commercial farms, directly measuring UN and FN is impractical meaning that quantification must be based on predictions rather than measured data. The purpose of this study was to use a statistical approach to evaluate the Cornell Net Carbohydrate and Protein System's (CNCPS) ability to predict N excretion in lactating dairy cows, and to compare CNCPS predictions to other relevant equations from the literature. Data to evaluate model predictions were compiled from published studies (n=32) that completed total collection N balance evaluations. Considerable care was taken to ensure that the treatments included in the data set (n=104) accounted for >90% of the N intake (NI), and used sound experimental methodology. Unaccounted N for the compiled data set was 1.47% ± 4.60% (mean ± SD). The results showed FN predictions could be improved by using a derivative of an equation proposed by Marini et al. (2008): FN (g/day) = (((NI (g/kg organic matter) × (1 – 0.842)) + 4.3) × organic matter intake (kg/day)) × 1.20, which, when evaluated against the compiled N balance data, had a squared coefficient of determination based on a mean study effect (R^2_{MP}) of 0.73, concurrent correlation coefficient (CCC) of 0.83 and a mean square prediction error (MSPE) of 781. Urinary N is currently over-predicted by the CNCPS due to inconsistencies in N accounting within the model. Incorporating the more accurate FN prediction into the current CNCPS framework and correcting the calculation error

considerably improved UN predictions (MSPE = 970, R^2_{MP} = 0.86, CCC = 0.90). The changes to FN and UN translate into an improved prediction of total manure N (MSPE = 623, R^2_{MP} = 0.96, CCC = 0.97) and have been incorporated into the latest version of the CNCPS (v6.1).

3.2 Introduction

Dairy producers in the U.S. are currently under pressure to use production systems that are more cost-efficient and have a smaller environmental footprint. Central to this debate is the management of nutrients, such as N and P, due to their key roles in ground- and surface-water pollution (Dou et al., 1998). Nitrogen is of particular concern on farms, not only because of its effects on water quality, but also because of the subsequent release of gases such as ammonia and nitrous oxide to the atmosphere (Fenn et al., 2003). To date, there has been no direct cost associated with the amount of N excreted by farms in North America. However, certain European countries (Belgium, Denmark, France and the Netherlands) tax farmers based on the amount of N excreted on the farm (Jones and OECD, 2004). This type of regulation could occur in the U.S. and will shift the nutritional goals on many farms (NRC, 2003).

To comply with future environmental standards, robust methods that quantify N outputs from dairy farms will be required (NRC, 2003). Directly measuring urinary N (UN) and fecal N (FN) on commercial farms is impractical meaning quantification must be based on predictions rather than measured data. The Cornell Net Carbohydrate and Protein System (CNCPS) is a mathematical model designed to evaluate the nutrient requirements of cattle over a wide range of environmental, dietary, management and production situations. The CNCPS also includes estimates of N and P excretion enabling integration with whole farm nutrient management plans

(Fox et al., 2004; Tylutki et al., 2008). An important constraint imposed during the development of the CNCPS was that the inputs used must be routinely available on most farms so the CNCPS has broad relevance for both research purposes and commercial farming (Fox et al., 2004). Currently, the model predicts total manure N (MN) adequately but the ratio of UN to FN is under predicted (Fox et al., 2004). For the CNCPS to be used effectively in nutrient management plans, more accurate estimates of UN and FN are required. The purpose of this study was to evaluate the current CNCPS predictions for UN and FN excretion and compare these to other relevant equations from the literature.

3.3 Materials and Methods

3.3.1 Data set development

Data for this evaluation were compiled from published studies that completed total collection N balance trials on lactating dairy cows. Observed study data for N intake (NI), milk N, FN and UN were compared to CNCPS predictions using the beta version of CNCPS 6.1. Studies were selected that presented the dietary and animal information required to run a simulation in CNCPS; this included a description of housing conditions, milk yield, milk fat, milk protein, live weight, stage of lactation, and stage of pregnancy. If stage of pregnancy and stage of lactation were not given, CNCPS default values were used. Required dietary information included DMI and a description and chemical analysis of the ration fed for each treatment. Studies often provided chemical analyses for forages and the complete ration, but not for the concentrates. If concentrate composition was not given, ingredients were selected from the CNCPS feed library, and used without alteration. If an ingredient was not present in the CNCPS feed library its composition was taken from the NRC (2001). Some studies presented a chemical analysis of the complete ration, but not the forages;

in this case forage CP and NDF concentrations were back-calculated from the complete ration composition presented by the study, and concentrate compositions from the CNCPS feed library. The calculated CP and NDF concentrations were then compared to corresponding forages in the CNCPS feed library and the closest match was selected. Minor adjustments were subsequently made to CP and NDF to get an exact match.

Our objective was to evaluate the CNCPS and alternative models for their ability to partition MN into FN and UN. To avoid confounding the prediction of FN and/or UN, it was important that NI and milk N reported by the study were identical to that accounted for by the model. Minor CP adjustments generally had to be made to ensure ration CP reported by the study was the same as that entered into the CNCPS. It was assumed that most of the variation probably occurred in the forages rather than the concentrates so forage CP was generally adjusted. Conflicting data on milk yield and milk protein composition compared to milk N output were presented in some studies. Nitrogen balance trials were often run in conjunction with larger production trials. Cows may have decreased milk yield when housed in a metabolism stall for the N balance component of the study compared to free- or tie-stall housing in the production trial. Milk yield was, therefore, adjusted to ensure milk N output measured by the study was the same as that accounted for by the model. The relationship between observed and model calculated NI and milk N is shown in Figure 3.3.1.

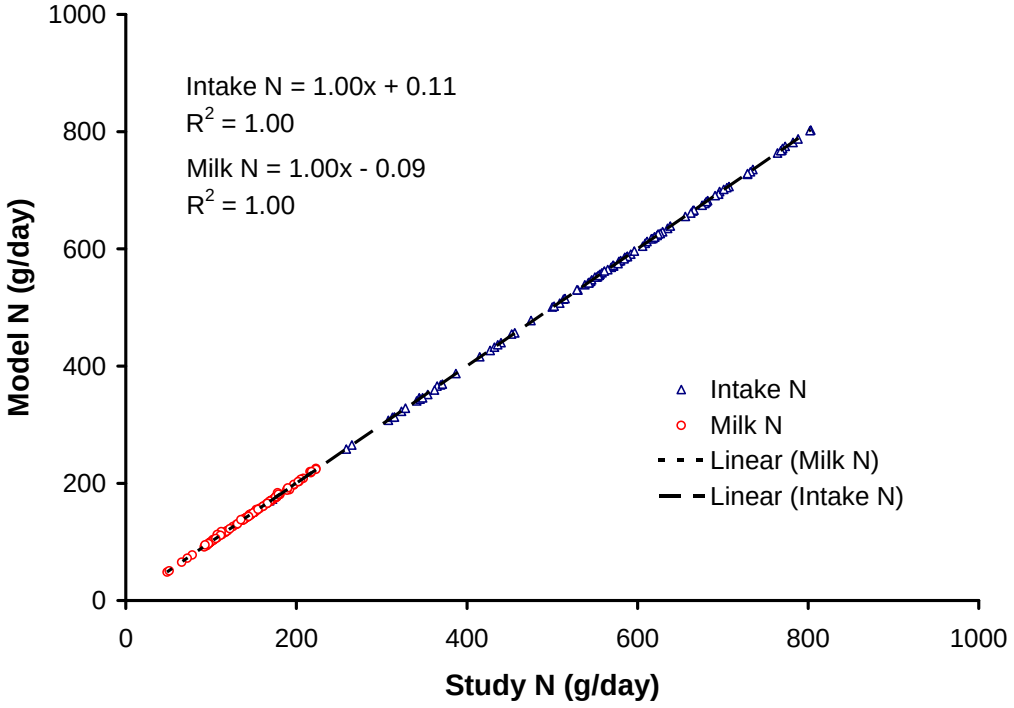

Figure 3.3.1: Comparison of milk and intake N measured by the compiled data set (32 studies, 104 treatments) and predicted by the model.

Nitrogen that could not be accounted for in the milk, feces or urine was generally reported by the study as retained N. Retained N was sometimes unrealistically high suggesting losses during the collection and/or analysis of feces and urine (Reynolds and Kristensen, 2008; Spanghero and Kowalski, 1997). Studies were omitted from the initial data set if retained or unaccounted N was above 10% in all of the treatments. Individual treatments were subsequently removed if unaccounted N was greater than 1 SD (5.9%) from the mean unaccounted N of the compiled data set. Adjustments were made to fecal N if samples were dried prior to analysis as per Juko et al. (1961). Consideration was also given to the laboratory method used to measure milk protein and scurf losses (Spanghero and Kowalski, 1997). Milk protein concentrations reported on a true protein basis were adjusted to CP basis and multiplied by a factor of

6.38 to give milk N while scurf losses were estimated as per Fox et al. (2004). Urinary N losses were considered minor given that all the studies acidified urine during collection (Spanghero and Kowalski, 1997). The resulting data set comprised 32 studies (Table 3.3.1), 104 treatments which were completed on 242 different cows. Unaccounted N for the whole data set was 1.5% ± 4.6% (mean ± SD). The range in dietary and animal characteristics is summarized in Table 3.3.2. A wide variety of forages were fed among treatments, and included corn silage (23%), alfalfa silage (12%), grass silage (27%), corn silage-alfalfa silage mix (27%), corn silage-grass silage mix (5%), and other (7%). 'Other' included fresh pasture, cereal silages and fresh red clover. The concentrate mixes represented were also diverse.

Table 3.3.1: Studies included in the data set used to evaluate model predictions.

(Beckman and Weiss, 2005)	(Jonker et al., 2002a)
(Birkelo et al., 2004)	(Knowlton et al., 2001)
(Brito et al., 2008)	(Martineau et al., 2007)
(Castillo et al., 2001)	(Moorby et al., 2009)
(Cherney et al., 2003)	(Noftsger and St-Pierre, 2003)
(Dinn et al., 1998)	(Petit and Tremblay, 1995)
(Drackley and Elliott, 1993)	(Raggio et al., 2004)
(Elliott et al., 1993)	(Ruiz et al., 2001)
(Erdman et al., 1982)	(Ruiz et al., 2002)
(Flis and Wattiaux, 2005)	(Schauff et al., 1992)
(Grieve et al., 1973)	(Van Dorland et al., 2007)
(Gruber et al., 1999)	(Wattiaux and Karg, 2004)
(Haig et al., 2002)	(Weiss and Wyatt, 2006)
(Holden et al., 1994)	(Wilkerson et al., 1997)
(Hristov et al., 2004)	(Wohlt et al., 1991)
(Jacobson et al., 1969)	(Wright et al., 2005)

Table 3.3.2: Descriptive statistics for dietary and animal characteristics from the compiled dataset used to evaluate model predictions (32 studies, 104 treatments).

	Mean	SD	Minimum	Maximum
Dietary characteristics				
DMI (kg/cow/day)	20.33	3.71	8.90	27.20
CP (% DM)	16.93	2.48	9.40	22.75
NDF (% DM)	32.49	5.92	16.65	43.22
Animal characteristics				
Full body weight (kg)	611.11	58.60	456.00	768.00
Milk yield (kg/day)	29.92	8.49	9.60	46.10
Milk fat (%)	3.64	0.44	2.50	4.87
Milk true protein (%)	2.87	0.21	2.29	3.36

3.3.2 Statistical analysis

A mixed model using the REML procedure of JMP (2007) was used to analyze the data using the model:

$$Y_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})X_{ij} + \varepsilon_{ij}$$

where:

- Y_{ij} = the expected outcome for the dependent variable Y observed at repetition j of the continuous variable X in study i,
- β_0 = the overall intercept across all studies,
- b_{0i} = the random effect of study i,
- β_1 = the overall slope of Y on X across all studies,
- b_{1i} = the random effect of study i on the slope of Y on X
- X_{ij} = the data associated with repetition j of the continuous variable X in study i, and
- ε_{ij} = random variation

The variance components in the model adhered to the following assumptions: $b_{0i} \sim N(0,\sigma^2_0)$, $b_{1i} \sim N(0,\sigma^2_1)$, and $\varepsilon_{ij} \sim N(0,\sigma^2_\varepsilon)$. Further information on mixed model methodology can be found in a review by St-Pierre (2001).

Current CNCPS equations for predicting FN and UN were compared to other relevant equations presented in the literature. The model fit for the current data set was also calculated and evaluated. Descriptions of the equations evaluated are presented in Table 3.3.3. During analysis, model predicted values were plotted on the X-axis, while the observed values were plotted on the Y-axis. This is because the model predicted values are deterministic and contain no random variation whereas the observed values contain natural variability (Tedeschi, 2006). Consequently, a β_1 that is greater than one represents an over-prediction while a β_1 that is less than one represents and under-prediction. To allow for the direct comparison of β_1 across different models, β_0 was forced through the origin. Theoretically this should have occurred naturally, and the range in the data meant that forcing it had little, if any effect on model outcomes. The squared sample correlation coefficients reported were based on either the best linear unbiased predictions (R^2_{BLUP}) or model predictions using a mean study effect (R^2_{MP}).

Additional model adequacy statistics were calculated to give further insight into the accuracy, precision and sources of error in each model (Tedeschi, 2006). Mean square prediction errors (MSPE) were used to indicate accuracy. A decomposition of the MSPE was also performed to give an estimation of the error due to central tendency (mean bias), regression (systematic bias), and random variation. Concordance correlation coefficients (CCC) were used to simultaneously account for accuracy and precision. Concordance correlation coefficients can vary from 0 to 1 with a value of 1 indicating no deviation from the Y = X line has occurred. Further description of these statistics is given by Tedeschi (2006).

Table 3.3.3: Source and description of the equations used to predict fecal N (FN; g/day) and urinary N (UN; g/day).

Equation	**Source**	**Description**[i]
FN CNCPS	(Fox et al., 2004)	FN = ((FEPB3 + FEPC + FEBCP + IDM) × 0.09)/6.25
FN 1	(Marini et al., 2008)	FN = ((NI (g/kg OM) × (1 – 0.842)) + 4.3) × OMI
FN 2	(Huhtanen et al., 2008)	FN = -21 + (DMI × 6.73) + (NI × 0.101)
FN 3[a]	Adapted from Marini et al. (2008)	FN = (((NI (g/kg OM) × (1 – 0.842)) + 4.3) × OMI) × 1.20
FN 4[a]	Adapted from Huhtanen et al. (2008)	FN = (-21 + (DMI × 6.73) + (NI × 0.101)) × 1.17
FN 5[b]	Current data set (Table 3.3.1)	FN = -21 + (DMI × 6.25) + (NI × 0.17)
UN CNCPS	(Fox et al., 2004)	UN = ((NI) − (SPA + (Milk × Milk CP × 10/0.93) + MP_{Preg} + MP_g + FN))/6.25
UN 1	(Huhtanen et al., 2008)	UN = -126 + (NI × 0.676)
UN 2	(Huhtanen et al., 2008)	UN = -91 + (Milk ×11.4)
UN 3	(Huhtanen et al., 2008)	UN = 27 + (NI ×0.844) + (DMI × -13)
UN 4	(Huhtanen et al., 2008)	UN = 40 + (NI × 0.879) + (DMI × - 9) + (Milk × - 3.9)
UN 5	(Nennich et al., 2006)	UN = (RDP × 0.0628) + 55.6

[a] Original equations from Marini et al. (2008) and Huhtanen et al. (2008) (FN 1 and 2, respectively) were adjusted to calibrate FN predictions to the current data set (32 studies, 104 treatments). The adjustments were based on the β1 coefficients derived from the mixed model analysis (Table 3.3.1).
[b] FN 5 = the regression equation derived from the current data set using DMI and NI as predictor variables.

[i] FEPB3 = Amount of feed B3 protein fraction in feces (g/day), FEPC = Amount of feed protein fraction C in feces (g/day), FEBCP = Amount of fecal bacterial protein (g/day), IDM = Indigestible dry matter intake (g/day), NI = Nitrogen intake (g/day), OMI = Organic matter intake (kg/day), DMI = Dry matter intake (kg/day), SPA = Requirement of net protein for scurf losses (g/day), Milk = Milk production (kg/day), Milk CP = Crude protein content of milk (%), MP_{Preg} = Metabolizable protein requirement for pregnancy (g/day), MP_g = Metabolizable protein requirement for gain (g/day) and RDP = Rumen degradable protein (g/day).

3.4 Results

3.4.1 Fecal nitrogen

Model adequacy statistics for FN and UN predictions are shown in Table 3.4.1. Among all 104 treatments, FN CNCPS predicted FN accurately with a slope of 1.00 and a MSPE of 884. The random effect of study in the mixed model analysis accounted for greater than 85% of the variation in predicted FN for every equation tested. Total variation was, therefore, explained with a great deal of precision as indicted by the high R^2_{BLUP} values. Variation attributed to the difference in slope among study, and the percentage of MSPE accounted for by systematic bias was either absent or trivial in every equation suggesting that, within study, the equations were very consistent. Equations FN 1 and 2, under-predicted FN by 20% and 17%, respectively, but both were more precise than the FN equation currently used in CNCPS (R^2_{MP} = 0.73 and 0.72, respectively). The high proportion of MSPE attributed to mean bias and the lack of error due to slope among study and systematic bias indicated these equations described the same biological relationship but differed in the total amount of FN predicted. Given this, FN 1 and FN 2 were adjusted to the current data set based on their corresponding β_1 coefficients (Table 3.4.1) and re-evaluated (FN 3 and 4, respectively). The adjusted equations were, both, more accurate and more precise than the FN equation used in CNCPS as indicated by higher R^2_{MP} values, lower MSPE, and higher CCC (Table 3.4.1). The equation developed from the current data set (FN 5 in Table 3.3.3) was comparable to FN 3 and 4, but slightly less accurate than FN 3 as indicated by the higher MSPE (787) and slightly higher proportion of error due to mean bias. Therefore, FN 3 was the strongest predictor of FN.

3.4.2 Urinary nitrogen

The total variation associated with predicting UN was higher than FN. None of the alternative equations were markedly better than the UN equation used in CNCPS which had the highest CCC (0.84) and lowest MSPE (1983). Every equation except UN 5 over-predicted UN. Currently the CNCPS calculates UN by difference (Table 3.3.3) making the accuracy of the FN prediction an important component in calculating UN. Given the performance of the UN equation in CNCPS (UN CNCPS) compared to alternatives, FN in the UN equation in CNCPS was replaced with FN 3 and this new UN equation was also tested (UN Proposed). The equation is described as follows:

$$\text{UN Proposed}^{ii} = ((NI) - (SPA + (Milk \times Milk\ CP \times 10/0.93) + MP_{Preg} + MP_g + (FN\ 3 \times 6.25)))/6.25$$

The results (Table 3.4.2) show UN Proposed had less total variation (MSE = 162.17) and a higher R^2_{MP} than UN CNCPS (0.86). Accuracy was also improved with a MSPE of 970 rather than 1983, and a slope of 0.93 rather than 0.86. The percentage of error due to model biases was 25% lower than UN CNCPS and the CCC increased from 0.84 to 0.90 indicating a more accurate and precise prediction of UN.

[ii] NI = Nitrogen intake (g/day), SPA = Requirement of net protein for scurf losses (g/day), Milk = Milk production (kg/day), Milk CP = Crude protein content of milk (%), MP_{Preg} = Metabolizable protein requirement for pregnancy (g/day), MP_g = Metabolizable protein requirement for gain (g/day) and FN 3 = Fecal N losses as predicted by equation FN 3 (g/day).

3.4.3 Manure and total nitrogen

The CNCPS calculates MN as the sum of FN and UN. The proposed changes to FN and UN translate into a more accurate prediction of MN (Table 3.4.2). Mean unaccounted study N was 1.5% for the whole data set which is similar to the 3% over-prediction of MN by MN Proposed (β_1 MN Proposed = 0.97). The MSPE was reduced from 998 to 623 and the proportion of error due to model biases was reduced by 25%. The increase in accuracy came at no cost to precision with both R^2_{BLUP} and R^2_{MP} remaining unchanged at 0.99 and 0.96, respectively. The CCC increased from 0.96 to 0.97 indicating a simultaneously accurate and precise model. Nitrogen accounting within the model was also tested for the current and proposed equations. Both had a slope of 1.00 and an R^2_{BLUP} and R^2_{MP} of 1.00, respectively. However, the intercept of TN CNCPS was -11.11g/day whereas TN Proposed was 1.38g/day. The intercept in TN should represent scurf losses meaning a negative value is unrealistic and suggests bias in N utilization within the model.

Table 3.4.1: Model adequacy statistics for the prediction of fecal N (FN; g/day) and urinary N (UN; g/day) from the CNCPS and alternative equations.

Equation	**Slope**[a]	**R^2_{BLUP}**[b]	**R^2_{MP}**[c]	**MSE**[d]	**Variance component**[e] **(%)**			**CCC**[f]	**MSPE**	**MSPE partitioned**[g] **(%)**		
					Study	Slope	Residual			U^M	U^S	U^R
FN CNCPS	1.00	0.98	0.69	109.74	85.19	0.04	14.77	0.80	884	1	1	98
FN 1	1.20	0.97	0.73	107.67	85.32	0.01	14.67	0.59	2307	64	3	33
FN 2	1.17	0.98	0.72	93.50	87.34	0.02	12.64	0.63	2052	60	2	38
FN 3	1.00	0.97	0.73	107.66	85.33	0.01	14.66	0.83	781	3	0	97
FN 4	1.00	0.98	0.72	93.50	87.34	0.01	12.65	0.83	810	4	0	96
FN 5	1.00	0.98	0.73	98.91	86.12	0.01	13.87	0.84	787	5	0	95
UN CNCPS	0.86	0.96	0.83	220.13	69.28	0.00	30.72	0.84	1983	28	36	35
UN 1	0.76	0.97	0.79	191.70	81.03	0.00	18.97	0.67	5579	55	29	15
UN 2	0.76	0.93	0.36	437.01	89.01	0.01	10.99	0.45	9087	34	37	29
UN 3	0.83	0.97	0.89	160.78	68.86	0.00	31.14	0.81	2365	61	21	19
UN 4	0.84	0.97	0.88	159.94	63.44	0.00	36.56	0.83	1985	62	14	24
UN 5	1.09	0.97	0.75	201.01	87.39	0.14	12.46	0.59	2035	6	43	52

[a] Slope of linear regression (intercepts were forced through the origin).
[b] R^2_{BLUP} = Squared sample correlation coefficient based on best linear unbiased predictions.
[c] R^2_{MP} = Squared sample correlation coefficient based on model predicted estimates.
[d] MSE = Mean square error.
[e] Percentage of variance related to the effect of study, differences in slope between study (study × prediction), and random variation.
[f] CCC = Concordance correlation coefficient.
[g] MSPE = Mean square prediction error, U^M = percentage of error due to mean bias, U^S = percentage of error due to systematic bias, U^R = percentage of error due to random variation. $U^M + U^S + U^R = 100$.

Table 3.4.2: Comparison of the adequacy of N predictions and accounting in the current CNCPS against proposed updates.

Equation[a]	**Slope**	**Intercept**[b]	$\mathbf{R^2_{BLUP}}$[c]	$\mathbf{R^2_{MP}}$[d]	**MSE**[e]	**Variance component**[f] **(%)**			**CCC**[g]	**MSPE**	**MSPE partitioned**[h] **(%)**		
						Study	Slope	Residual			U^M	U^S	U^R
FN CNCPS	1.00		0.98	0.69	109.74	85.19	0.04	14.77	0.80	884	1	1	98
FN 3	1.00		0.97	0.73	107.66	85.33	0.01	14.66	0.83	781	3	0	97
UN CNCPS	0.86		0.96	0.83	220.13	69.28	0.00	30.72	0.84	1983	28	36	35
UN Proposed	0.93		0.97	0.86	162.17	70.79	0.01	29.20	0.90	970	14	26	60
MN CNCPS	0.94		0.99	0.96	146.15	60.76	0.00	39.24	0.96	998	42	15	43
MN Proposed	0.97		0.99	0.96	154.14	56.82	0.00	43.17	0.97	623	8	24	68
TN CNCPS	1.00	-11.11	1.00	1.00	4.21	0.00	0.00	100.00	1.00				
TN Proposed	1.00	1.38	1.00	1.00	0.05	72.20	0.00	27.80	1.00				

[a] FN = fecal N, UN = urinary N, MN = manure N (FN +UN), TN = total N accounted for by the model (productive N + FN + UN).
[b] The intercept represents the difference between NI and MN+ productive N (g/day). No intercept means it was forced through the origin.
[c] R^2_{BLUP} = Squared sample correlation coefficient based on best linear unbiased predictions.
[d] R^2_{MP} = Squared sample correlation coefficient based on model predicted estimates.
[e] MSE = Mean square error.
[f] Percentage of variance related to the effect of study, differences in slope between study (study × prediction), and random variation.
[g] CCC = Concordance correlation coefficient.
[h] MSPE = Mean square prediction error, U^M = percentage of error due to mean bias, U^S = percentage of error due to systematic bias, U^R = percentage of error due to random variation. $U^M + U^S + U^R = 100$.

3.5 Discussion

The alternative equations from the literature evaluated in the current analysis (FN 1-4 and UN 1-5) were, themselves, all developed from large data sets and derived using mixed model statistical procedures to account for random error between studies. Simple linear regressions can lead to a misinterpretation of biological relationships (St-Pierre, 2001). Therefore, studies that did not use a mixed model analyses were not considered. The data set used for the evaluation encompassed a wide range of milk production, NI, DMI and ration compositions to ensure the assessment had relevance to a broad range of production systems. Considerable care was taken to ensure the treatments included in the data set used sound methodology, and accounted for greater than 90% of the NI in the milk, feces and urine. Nitrogen balance studies often overestimate N retention due to losses incurred during collection and analysis (Bockmann et al., 1996; Castillo et al., 2000; Reynolds and Kristensen, 2008; Spanghero and Kowalski, 1997). Providing the animals used in balance studies are fully grown, in mid- to late-lactation and not in the last trimester of pregnancy, intake N should be approximate to the amount of N lost from the body (Hegsted, 1976). Despite this, even the most carefully conducted N balance studies are unable to account for all the intake N resulting in an overestimation of retained N (Spanghero and Kowalski, 1997). Edits were made to the current data set to account for scurf N losses, FN losses when feces were dried prior to analysis, and differences in milk N based on the analytical method used (see section 3.3). The editing process reduced unaccounted N from 5.1% to 1.5% of NI which is lower than comparable data sets from Spanghero and Kowalski (1997) and Bockman et al. (1996) who reported unaccounted N of 4.4% and 6.0% of NI, respectively. It must be noted that error in the chemical analysis of the ration fed and DMI measurements may also contribute to error in N balance estimates which is not often mentioned.

The current CNCPS framework relies on a combination of measured inputs and empirical predictions to account for N usage within the animal. Urinary N is calculated as the difference between NI and the sum of N accretion, milk N, N retained by the conceptus, scurf N and FN (Table 3.3.3). This means a misrepresentation of any of these factors will cause a subsequent error in UN. Having the model structured in this way essentially pools the input error from other parameters into UN which may be a reason for the large portion of the MSPE in UN CNCPS being accounted for by model biases (Table 3.4.1). It also makes the accuracy of dietary and animal inputs critical in predicting both MN and the partitioning of FN and UN. From a modeling standpoint, structuring the model in this manner enables N to first be accounted for from parameters that are routinely measured (DMI, milk), and then from parameters that are easily predicted i.e. maintenance, pregnancy and growth (Fox et al., 2004; NRC, 2001; Tylutki et al., 2008). This leaves a smaller pool of N that needs to be empirically predicted, and translates the accuracy of other, more robust measurements, to the prediction of more variable parameters. The random effect of study in the mixed model analysis accounted for a high proportion of variation in both FN and UN predictions and resulted in high R^2_{BLUP} values (Table 3.4.1 andTable 3.4.2, respectively). In practice, R^2_{BLUP} can be misleading as random 'farm to farm' variation can not be accounted for given that there are no measured values to compare model predictions to. Consequently, R^2_{MP} values were also presented which use an average study effect across the whole data set and give a better indication of the amount of variation the model may explain in the practical situation.

Fecal N is calculated in the CNCPS as the residual from the digestion of B3 and C protein fractions, bacterial losses and losses from protein bound to the indigestible DM (IDM; Table 3.3.3). Compared to observed values, the current FN equation in CNCPS

predicted FN accurately (slope = 1.00, MSPE = 884) which is in contrast to the suggestions of Fox et al. (2004) that, due to some of the metabolic N losses and IDM also being accounted for in the bacterial N fraction, the equation would over-predict FN. Huhtanen et al. (2008) showed that bivariate equations including both DMI and NI as independent variables are able to predict FN more precisely than univariate models that are simple functions of DMI or NI. Biologically, metabolic and endogenous N is related to DMI, whereas undigested feed N is related to NI meaning the bivariate model is able to account for the origin of each of the FN sources more correctly (Huhtanen et al., 2008; Marini et al., 2008). Marini et al. (2008) also found that the fermentation rate of dietary NDF has an important effect on determining endogenous N losses. Increasing levels of NDF appeared to reduce digested N, however, rapidly fermenting carbohydrates increased total tract N digestion. Carbohydrates that ferment more slowly can reach the hindgut and provide an energy source for microbes that trap N, and are subsequently excreted in the feces (Dewhurst and Thomas, 1992; Tamminga, 1992; Van Soest, 1994). Both FN 1 and 2 calculated FN based on functions of NI and DMI (FN 2) or OMI (FN 1) and were able to account for FN more precisely than FN CNCPS. However, both equations considerably under-predicted total FN. Interestingly, the systematic bias associated with FN 1 and 2 was low, indicating that the biological relationship between the two equations was similar. The large mean bias and subsequent under-prediction of FN could be explained by simple differences in the level of unaccounted N from the N balance data used to derive the equations. Adjusting FN 1 and 2 based on the level of under prediction (FN 3 and 4) resulted in more accurate and precise predictions of FN than the FN equation in CNCPS. The relationship of FN excretion from the current data set (FN 5) was comparable to that developed by Huhtanen et al. (2008). However, both equations resulted in a negative intercept which is biologically impossible. Huhtanen et al.

(2008) attempted to solve this problem by using a quadratic model but found the linear model to be more precise. Another alternative is to predict FN per unit of DMI or OMI as presented by Marini et al. (2008), which, when calibrated to the current data set (FN 3), resulted in the most accurate and precise prediction of FN tested (R^2_{MP} = 0.73, MSPE = 781).

The majority of excess N not utilized for growth or production is excreted in the urine, regardless of whether it is absorbed as AA or simply ammonia (Broderick, 2007). True endogenous N, or maintenance losses are the only real constant source of UN and account for approximately 0.35 g N/kg of metabolic weight per day (Dewhurst and Thomas, 1992). Other more variable sources include rumen degradable N not incorporated into microbial protein, metabolites of microbial nucleic acids, and products of the incomplete utilization of absorbed AA (Tamminga, 1992). Many of the equations used to predict UN in the literature use functions of MUN because it has a direct relationship to UN and is relatively easy to measure (Burgos et al., 2007; Jonker et al., 1998; Kauffman and St-Pierre, 2001; Nennich et al., 2006). However, using MUN to predict UN in the CNCPS would mean an additional input, without which, UN could not be predicted. This may limit the usefulness of the CNCPS as a nutrient management tool and adds an additional source of variation from the analysis of MUN (Arunvipas et al., 2004; Peterson et al., 2004). Predictions based on dietary parameters and/or milk production (UN 1-5) were less accurate than UN CNCPS which, despite over-predicting UN by 14%, was the best predictor of UN tested (CCC = 0.84). The over-prediction in UN CNCPS could be explained by an error in the UN calculation within the model where a portion of productive N is not subtracted from the UN equation which results in a pool of N being accounted for twice (TN CNCPS; Table 3.4.2). The data set used to derive UN 1-4 estimated UN based on the difference

between NI, milk N and FN (Huhtanen et al., 2008). The analysis of FN 2 indicated that unaccounted FN in the data set of Huhtanen et al. (2008) was 17% higher than the current data set which explains the over-prediction of UN from UN 1-4. In contrast, the data set used to derive UN 5 was based on UN measurements from N balance studies (Nennich et al., 2006) so the 9% over-prediction is probably due to differences in the level of unaccounted N. Given the difficulties in establishing a robust data set to derive UN predictions from, the UN equation in CNCPS was re-derived (UN Proposed) using FN 3 to predict FN outputs. The resulting equation (UN Proposed) still over-predicted UN by 7% but this corresponded more closely to the 1.5% total unaccounted N in the current data set while the accuracy and precision of UN predictions was considerably improved (Table 3.4.2). The improvement in the FN and UN predictions improved the 6% over-prediction of the MN estimates in CNCPS and aligned MN more closely to the current data set (MN Proposed). Implementing these changes would result in a more accurate and precise prediction of UN, FN and MN and improve the usefulness of the CNCPS as a nutrient management tool.

3.6 Conclusions

To comply with future environmental standards, robust methods that quantify N outputs from dairy farms are required. The results of this study show the prediction of fecal, urinary and total manure N can be improved through the adoption of a new FN equation, and a reconstruction of the UN equation to account for bias in N utilization within the model. These changes have been incorporated into latest version of the CNCPS (v6.1).

CHAPTER 4: USING THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM AS A TOOL TO IMPROVE NITROGEN UTILIZATION IN COMMERCIAL DAIRY HERDS

4.1 Abstract

Nitrogen utilization is becoming a central component in ration balancing as farmers try to maximize milk protein yields, decrease feed costs and conform to modern environmental standards. Feeding excess CP can result in unnecessary feeding expenses with no return in milk or milk protein yield in addition to having important environmental consequences. Recent changes to the Cornell Net Carbohydrate and Protein System (CNCPS) have resulted in increased model sensitivity to N intake allowing rations to be developed with reduced environmental impact. Farm level studies are required to evaluate the updated model on commercial farms. In this study, the CNCPS (v6.1.18) was used to adjust the diets of two commercial herds in western NY to improve N utilization and reduce feed costs while maintaining high levels of milk production (Part 1). In addition, thirteen herds that were producing high levels of milk (mean = 87 lbs/cow/day) on low CP diets (14.3-16.5 % DM) were characterized as examples of attainable N utilization targets (Part 2). In part 1, CP was reduced by approximately 1% DM, MUN was decreased by approximately 2 mg/dl and income over feed cost was improved on both farms. Part 2 showed that high milk and milk protein yields can be achieved on diets supplying less than 16% CP and that N use efficiency can be as high as 38%. This study confirms that the updated CNCPS can be successfully used to develop diets that enhanced N use efficiency under the constraints of a modern commercial dairy farm.

4.2 Introduction

The high value of milk protein, increasing feed costs, and growing concerns for the environment has made N utilization a central component in ration balancing. Feeding excess CP can result in unnecessary feed expenses with no return in milk or milk protein yield. Furthermore, the majority of excess dietary N is excreted in the urine which is the most environmentally labile form (Broderick, 2003). On the other hand, shorting the cow of AA will limit milk protein yield and revenue, which can be even more expensive than overfeeding (VandeHaar and St-Pierre, 2006). Balancing the cow's metabolizable protein requirements with correct quantities of RDP and RUP, while not overfeeding, will have positive effects not only on ration cost and profitability, but also the environment (Kalscheur et al., 1999).

Improving the efficiency of nutrient use requires accurate predictions of how various feed fractions behave as they flow through the digestive tract. The Cornell Net Carbohydrate and Protein System (CNCPS) is an extensive mathematical model designed to evaluate the nutrient requirements of cattle over a wide range of environmental, dietary, management and production situations. Recent evaluations by Lanzas et al. (2007) have suggested that the way the CNCPS characterizes feed proteins and their associated degradation and passage rates may cause protein to be overfed. Van Amburgh et al. (2007) provides a detailed description of recent changes that have been made to improve CNCPS predictions including a re-characterization of various pool constituents, degradation rates and, passage rate assignments. The result is a model that is more sensitive to N intake and can be used to develop cost-effective rations that result in lower N excretion (Van Amburgh et al., 2007). The changes represent an ongoing effort to improve the CNCPS as new data become available, and the understanding of biological mechanisms improve (Van Amburgh et al., 2007).

However, field based studies are required to evaluate the efficacy of the updates to the industry. The overall objective of this study is to evaluate N utilization on commercial dairies using the updated version of CNCPS (v6.1). The specific objectives are:

- Use the CNCPS to re-balance existing diets of two commercial herds in a way that maintains milk production, but reduces N excretion (Part 1).

- Characterize thirteen farms that are currently producing high levels of milk and feeding low protein diets as examples of reachable targets (Part 2).

4.3 Materials and Methods

4.3.1 Part 1 – Improving N utilization in commercial herds

Two progressive dairy nutritionists from NY State were approached to participate in Part 1. Each was asked to select a herd with high milk production (>80 lbs/cow/day), consistent management but with an opportunity to reduce the level of protein intake in the herd. Farms A and B were chosen based on their willingness to participate and suitability to the study. Basic information on each farm is shown in Table 4.3.1.

Table 4.3.1: Basic information for two commercial farms in western New York.

	Farm A	**Farm B**
Number of milking cows	400	600
Ration	TMR	TMR
Source of forages	Home grown	Home grown
bST use	Eligible cows	Eligible cows
Milking regime	2X[a]	2X
Housing	Free stall	Free stall

[a] 2X = cows are milk twice-a-day.

The study ran from Sep-08 to Apr-09 for Farms A and B, respectively. Farm visits were made approximately once every two months, but regular contact was maintained

with the farms nutritionist (fortnightly) who provided information on the level of milk production, DMI and any additional changes of relevance to the study (forage changes, concentrate changes). Initial farm visits were used to collect the data required to model the farm in CNCPS (v6.1) including a complete description of the diet, forage analyses, DMI, milk production, milk fat, milk true protein, full body weight of the cows, body condition scores, stage of lactation, days pregnant and a description of the environment and facilities. Subsequent visits were used to discuss diet changes with the farm, collect forage samples and view the herd. Both farms were monitored for approximately two months prior to any dietary changes. Dietary changes were made as required to meet the study objectives, but also in response to changes in forage composition as different sections of the bunk silos were fed. New diets were developed using the CNCPS. Ration changes were implemented only after approval was obtained from both the farms nutritionist and farm managers.

Bulk tank MUN concentration was monitored as an independent indicator of protein utilization. In addition to bulk tank data, individual cow data on milk yield, milk fat, milk protein and MUN were collected using monthly DHIA testing (Dairy One, Ithaca, NY). Farm A ran 3 milk cow groups (Pens 1, 2 and 3). Pen 1 was fresh cows, pen 2 high producing cows ("high group") and pen 3 low producing cows ("low group"). The same grain mix was fed to every group, however, the ratio of corn silage to haylage and proportion of grain in the diet varied between groups. In this study, the high group (pen 2) was used to base dietary changes on. Data presented on milk yield, fat, protein and MUN from DHIA testing are weighted means of pens 1, 2 and 3. Estimates of N utilization, feed costs, income, and ration composition correspond to pen 2. Farm B ran 4 milk cow groups (pens 1, 2, 3 and 8). Pens 1 and 8 were first lactation cows and Pens 2 and 3 were mature cow. All cows were fed the same ration.

Data presented are weighted means of these four groups. Bulk tank data represent whole herd averages and include additional cows that were not present in the DHIA data.

Feed prices were based on a consultant's price list for the week of 4/20/2009. Milk income was calculated from the April-09 federal order price. The producer price differential used was based on Buffalo, NY and adjustments were made to account for changes in fat, protein and other solids. Dry matter intake data for Farm A were estimated based on refusals while Farm B used TMR Tracker (Digi-Star, Fort Atkinson, WI). Data presented represent the mean for each month of the study. Estimations of urinary, fecal and total manure N outputs were based on the findings of Chapter 2.

4.3.2 Part 2 – Characterizing commercial herds with a high N utilization

Dairy nutritionists and producers from three states (NY, WI and PA) were approached in the spring of 2009 to participate in Part 2 of this study. The thirteen farms included in the study were producing high levels of milk (86.7 ± 11.3 lbs/cow/day; mean ± SD) and milk protein (3.1 ± 0.1 %; mean ± SD) with ration CP levels of 14.3-16.5 %. Adequate information was supplied on each farm to run a simulation in CNCPS v6.1 (see section 4.3.1). Bulk tank MUN concentrations were also provided to give an indication of N utilization independent to CNCPS predictions. Farms 4 and 5 provided multiple rations. Farm 4 was feeding 3 different rations to the herd based on specific group requirements (Ration D = fresh cows, Ration E = high cows and Ration F = low cows), while farm 5 fed different diets in January and May (Rations G and H, respectively). All other farms fed one ration to the lactating cows. Farms 4 and 7

provided additional information on daily bulk tank MUN concentrations from December 08 to May 09. All other data presented are values from CNCPS outputs.

4.4 Results and Discussion

4.4.1 Part 1 – Improving N utilization in commercial herds

The following figures and tables summarize the changes observed on Farm A and B over the course of study. Key points relating to the changes are subsequently discussed.

Farm A

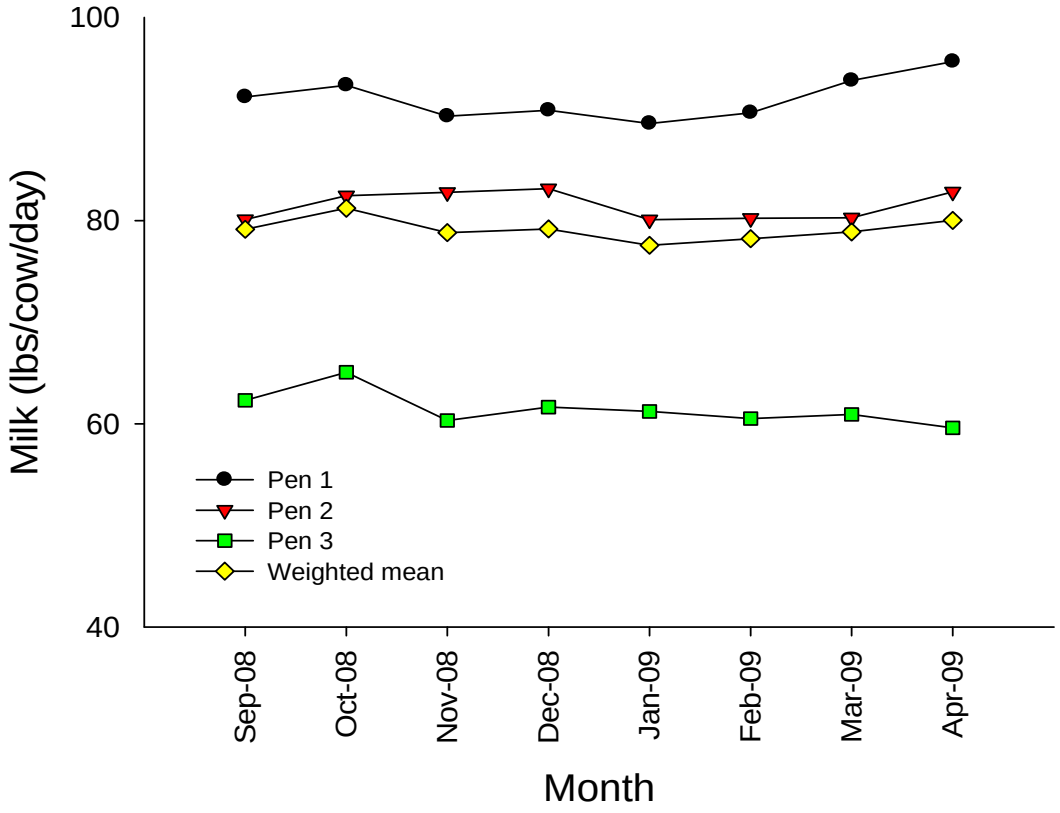

Figure 4.4.1: Mean monthly milk production (weighted mean and individual pens) for pens 1, 2 and 3.

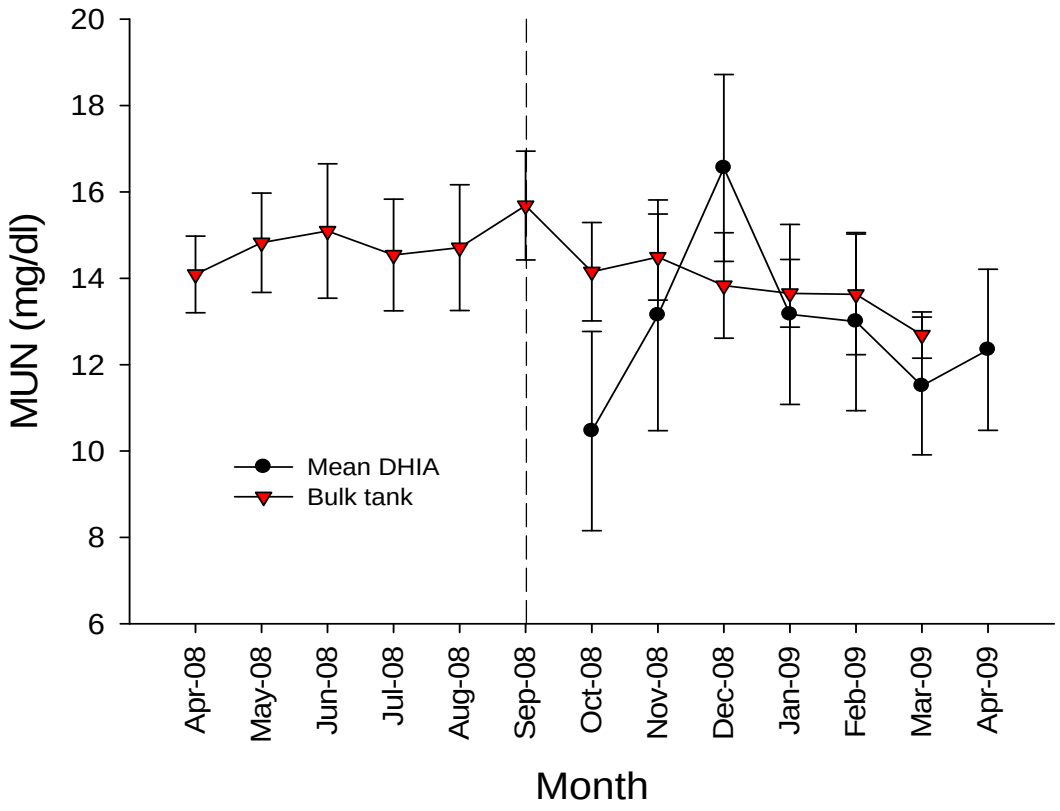

Figure 4.4.2: Mean milk urea N from bulk tank (Apr 08-09) and DHIA (Oct-08 to Apr-09) testing. The dashed line represents the start of the study. Bulk tank represents the whole herd average; mean DHIA represents the weighted mean of pens 1, 2 and 3.

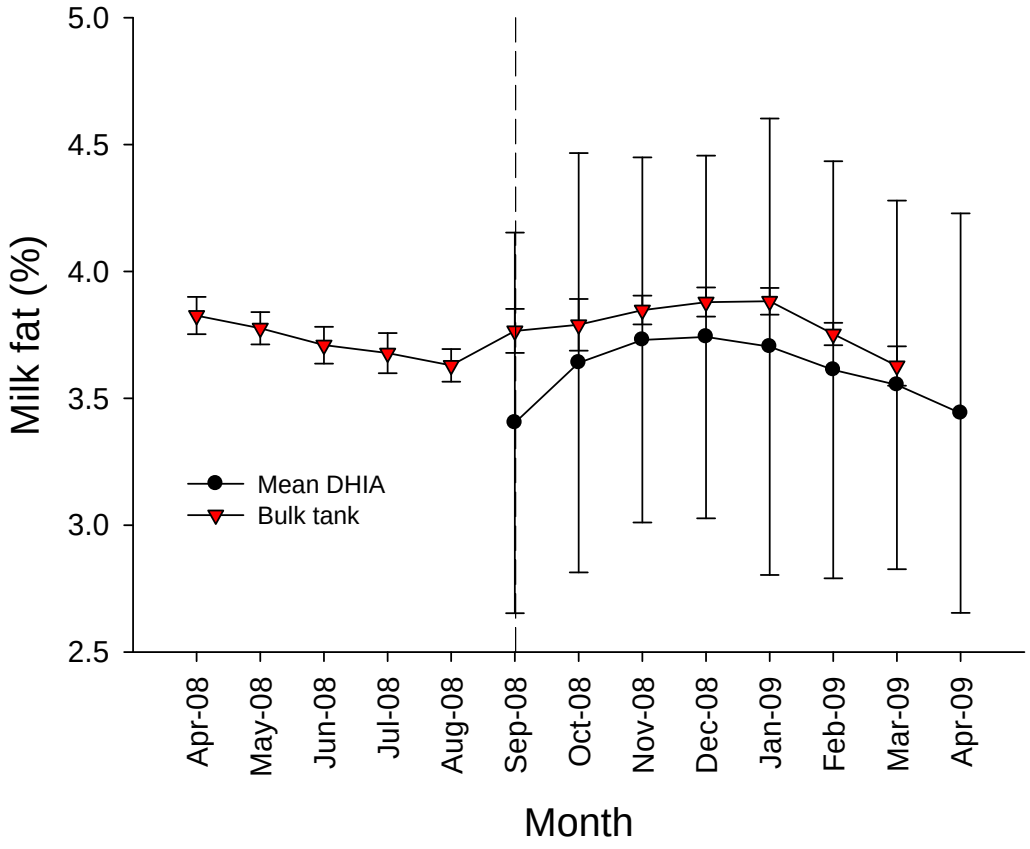

Figure 4.4.3: Mean milk fat from bulk tank (Apr 08-09) and DHIA (Sep-08 to Apr-09) testing. The dashed line represents the start of the study. Bulk tank represents the whole herd average; mean DHIA represents the weighted mean of groups 1, 2 and 3.

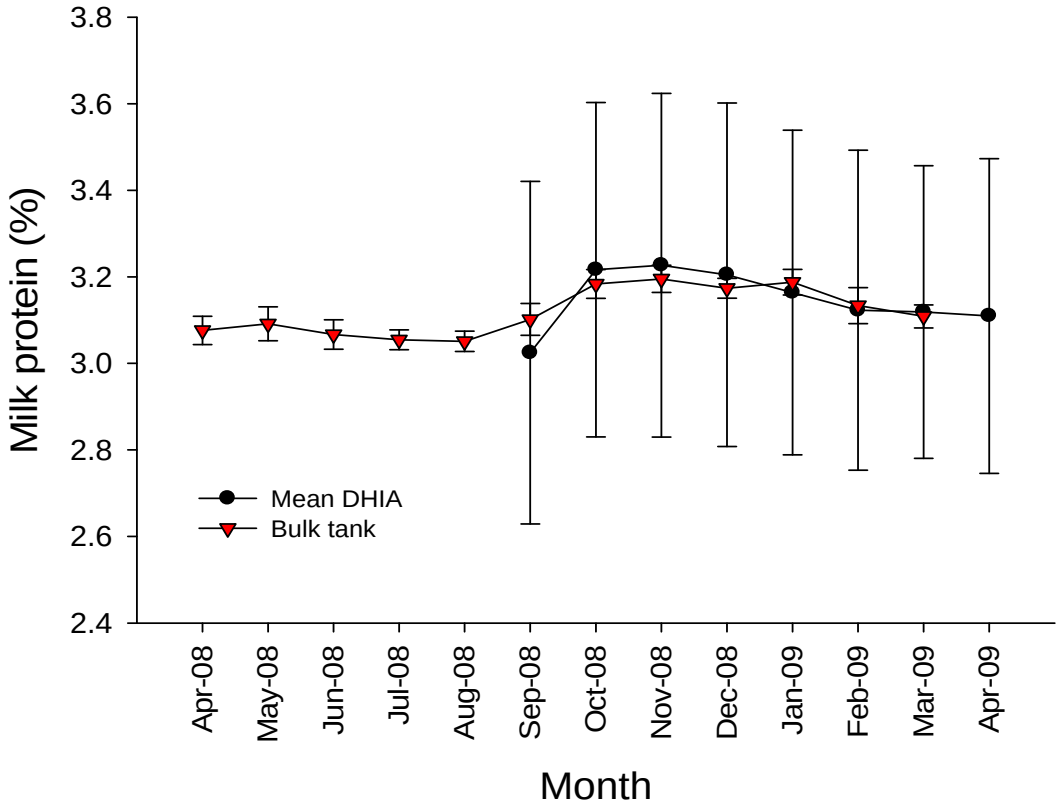

Figure 4.4.4: Mean milk protein from bulk tank (Apr 08-09) and DHIA (Sep-08 to Apr-09) testing. The dashed line represents the start of the study. Bulk tank represents the whole herd average; mean DHIA represents the weighted mean of groups 1, 2 and 3.

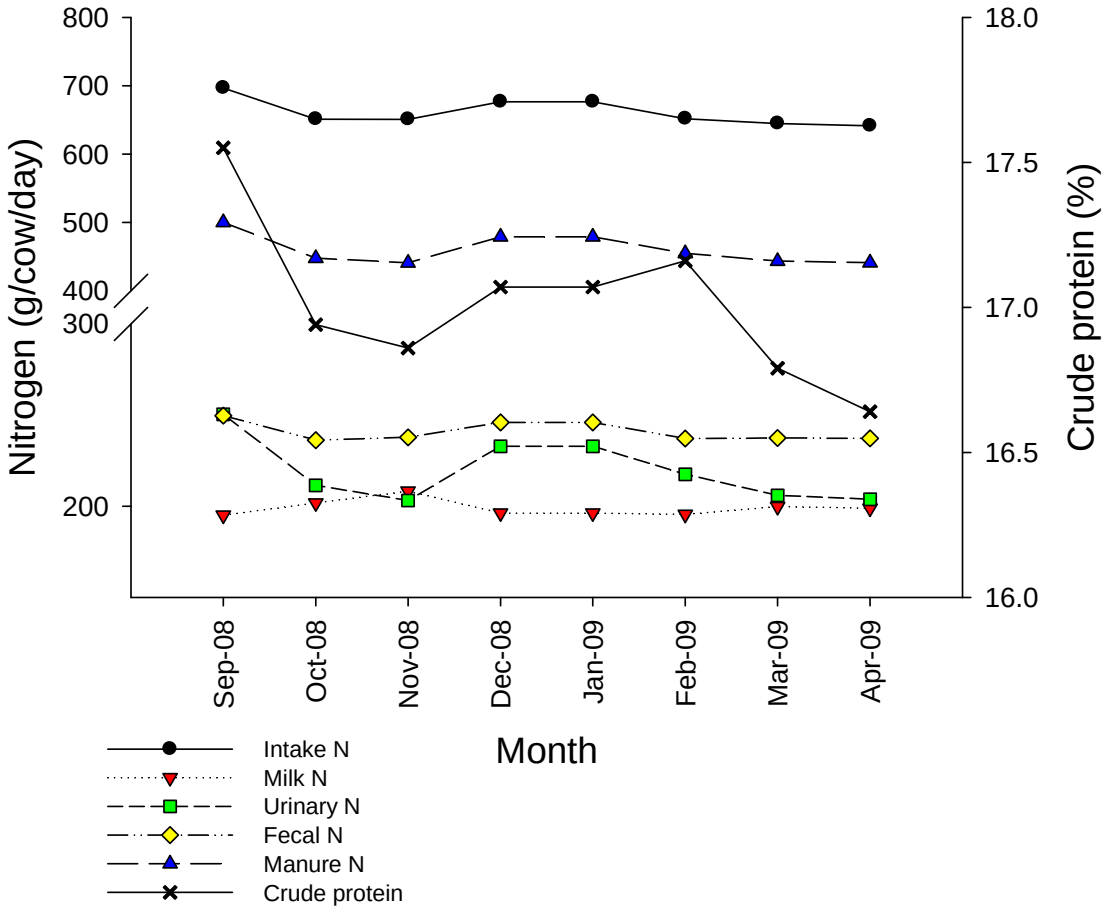

Figure 4.4.5: Mean N balance and ration CP for the high group (pen 2) from Sep-08 to Apr-09.

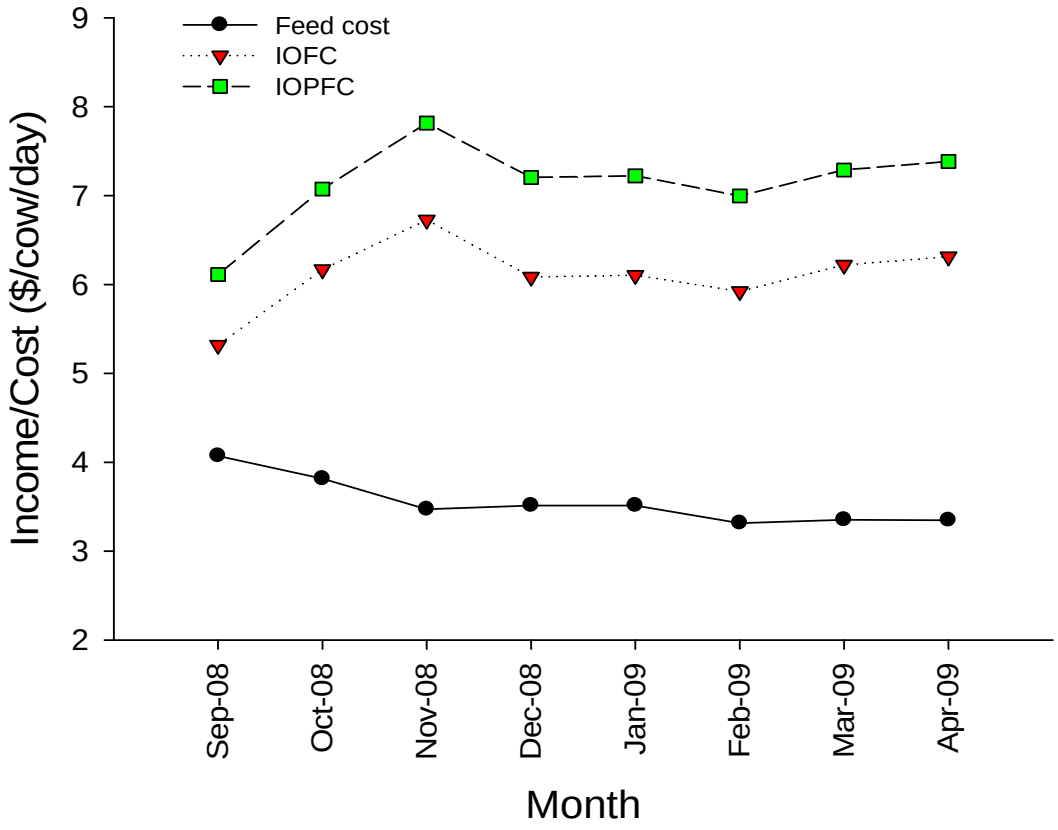

Figure 4.4.6: Mean feed cost, income over feed cost (IOFC) and income over purchased feed cost (IOPFC) for the high group from Sep-08 to Apr-09.

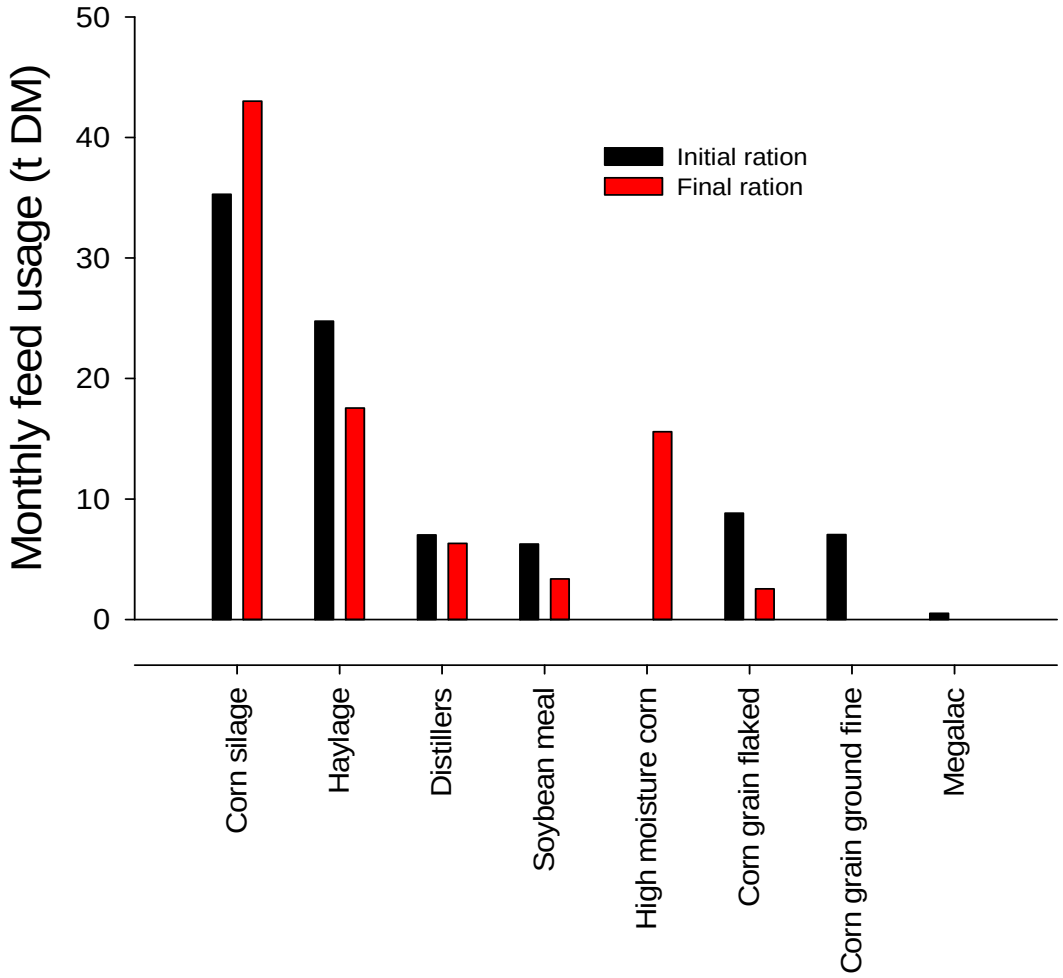

Figure 4.4.7: Difference in the use of specific ration ingredients each month for pen 2 (130 cows; refer to Figure 4.4.1 for milk production) from the initial ration to the final ration. Ration changes are summarized in Table 4.4.1.

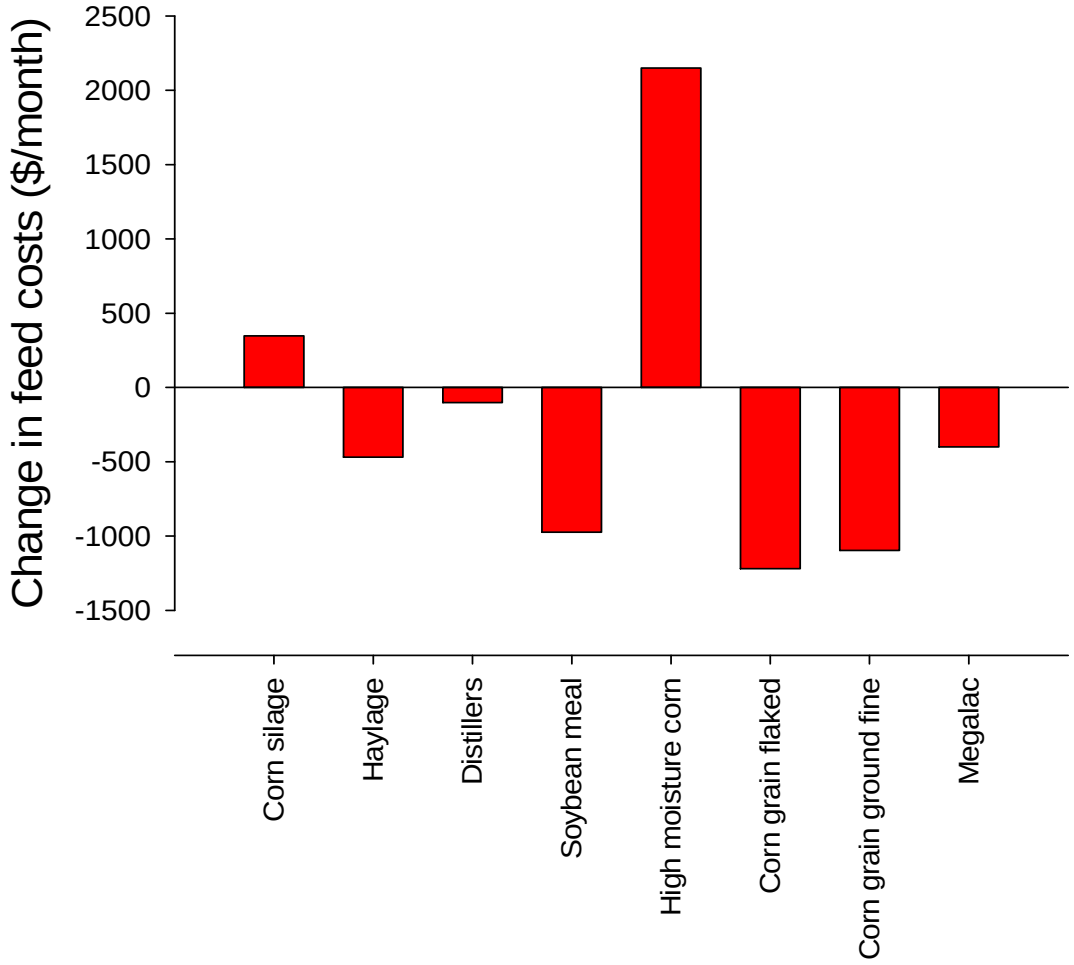

Figure 4.4.8: Difference in the amount spent each month on various ration ingredients for the high group (130 cows) from the initial ration to the current ration.

Table 4.4.1: Ration composition over the trial period for the high group (pen 2) on Farm A.

Ingredient (lbs/cow/day)	Sep-08	Oct-08[a]	Nov-08	Dec-08	Jan-09	Feb-09	Mar-09[b]	Apr-09[c]
Corn silage	17.51	16.97	17.89	19.38	19.38	19.28	20.96	21.34
Haylage	12.28	12.00	12.38	12.01	12.01	10.53	8.70	8.70
Distillers	3.48	3.45	3.03	3.10	3.10	3.11	3.17	3.13
Soybean meal	3.10	1.90	2.04	2.09	2.09	1.66	1.72	1.67
High moisture corn	0.00	3.38	7.14	7.31	7.31	7.48	7.83	7.73
Soybean hulls	1.80	1.78	1.63	1.63	1.63	1.54	1.60	1.59
Corn gluten feed dry	1.80	1.90	1.81	1.82	1.82	1.75	1.80	1.80
Molasses cane	0.24	0.23	0.22	0.22	0.22	0.21	0.22	0.22
Wheat midds	1.33	2.02	1.59	1.60	1.60	1.51	1.56	1.55
Corn grain flaked	4.38	2.97	1.23	1.23	1.23	1.22	1.24	1.26
Corn grain ground fine	3.49	1.71	0.00	0.00	0.00	0.00	0.00	0.00
Corn gluten meal	0.28	0.28	0.28	0.28	0.28	0.26	0.26	0.26
Soy pass	1.34	1.37	1.29	1.29	1.29	1.22	1.26	1.26
Sugar	0.00	0.26	0.28	0.28	0.28	0.28	0.28	0.29
Blood meal	0.73	0.66	0.67	0.67	0.67	0.66	0.69	0.68
Dairymans edge	0.00	0.09	0.09	0.09	0.09	0.08	0.08	0.08
Nugget	0.00	0.25	0.28	0.28	0.28	0.27	0.28	0.27
Bakery by products	0.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Megalac	0.25	0.26	0.00	0.00	0.00	0.00	0.00	0.00
Minerals and vitamins	1.58	1.53	1.34	1.34	1.34	1.27	1.28	1.28
Intake (lbs DM/cow/day)	54.49	53.26	52.64	53.19	54.62	52.33	52.97	52.83

[a] Changed from 07 to 08 Corn silage, changed from 1st to 2nd cutting haylage and the first experimental diet was fed.
[b] Haylage changed to 50% 2nd cutting and 50% 3rd cutting.
[c] Haylage changed to 100% 3rd cutting.

Farm B

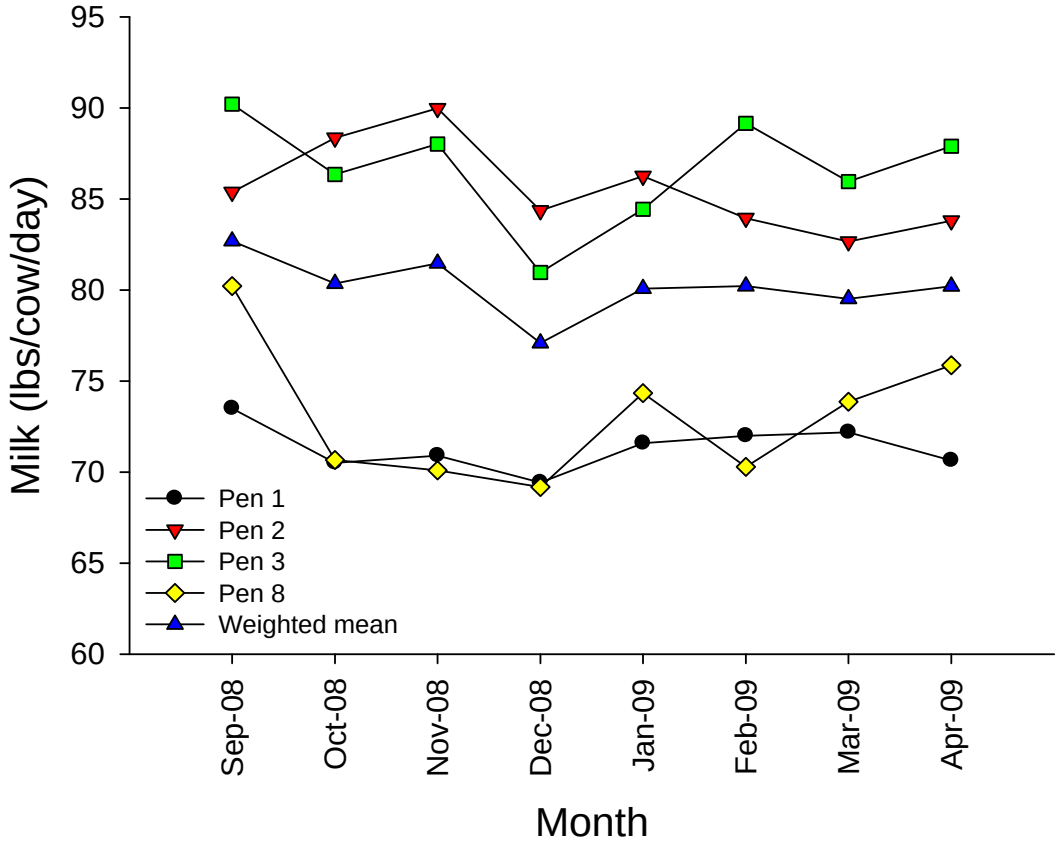

Figure 4.4.9: Mean monthly milk production (weighted mean and individual pens) for pens 1, 2, 3 and 8.

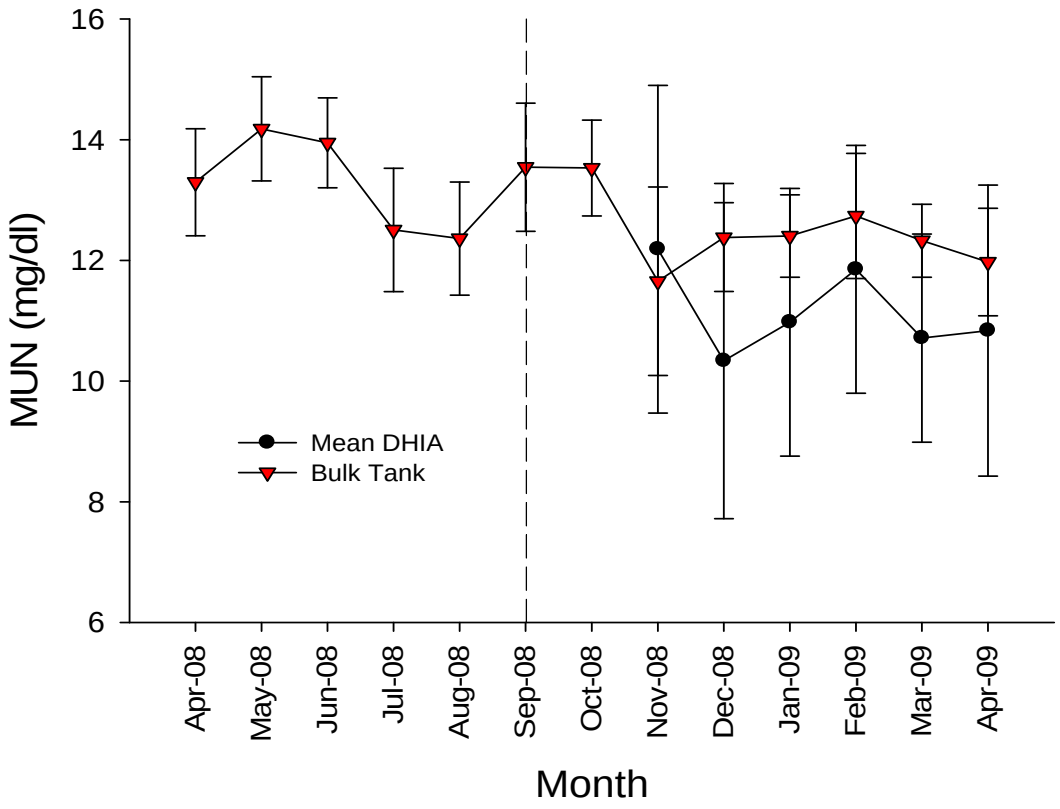

Figure 4.4.10: Mean milk urea N from bulk tank (Apr 08-09) and DHIA (Nov-08 – Apr-09) testing. The dashed line represents the start of the study. Bulk tank values represent the whole herd average; mean DHIA represents the weighted mean of pens 1, 2, 3 and 8.

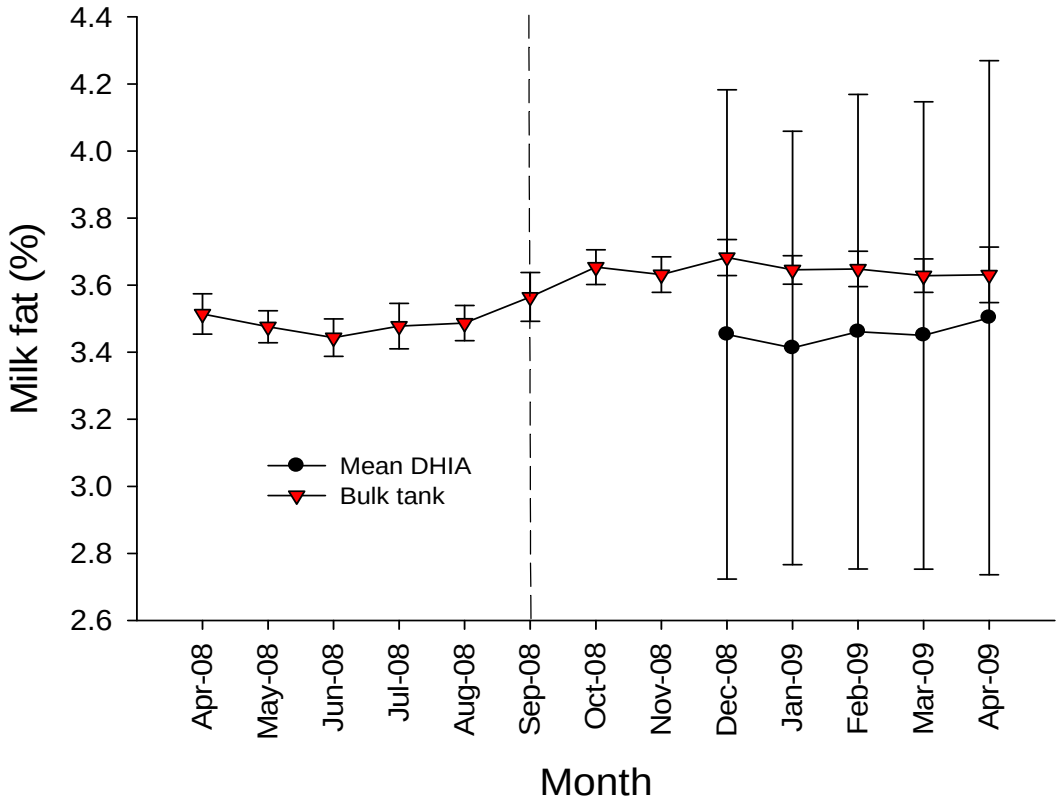

Figure 4.4.11: Mean milk fat from bulk tank (Apr 08-09) and DHIA (Dec-08 to Apr-09) testing. The dashed line represents the start of the study. Bulk tank values represent the whole herd average; mean DHIA represents the weighted mean of pens 1, 2, 3 and 8.

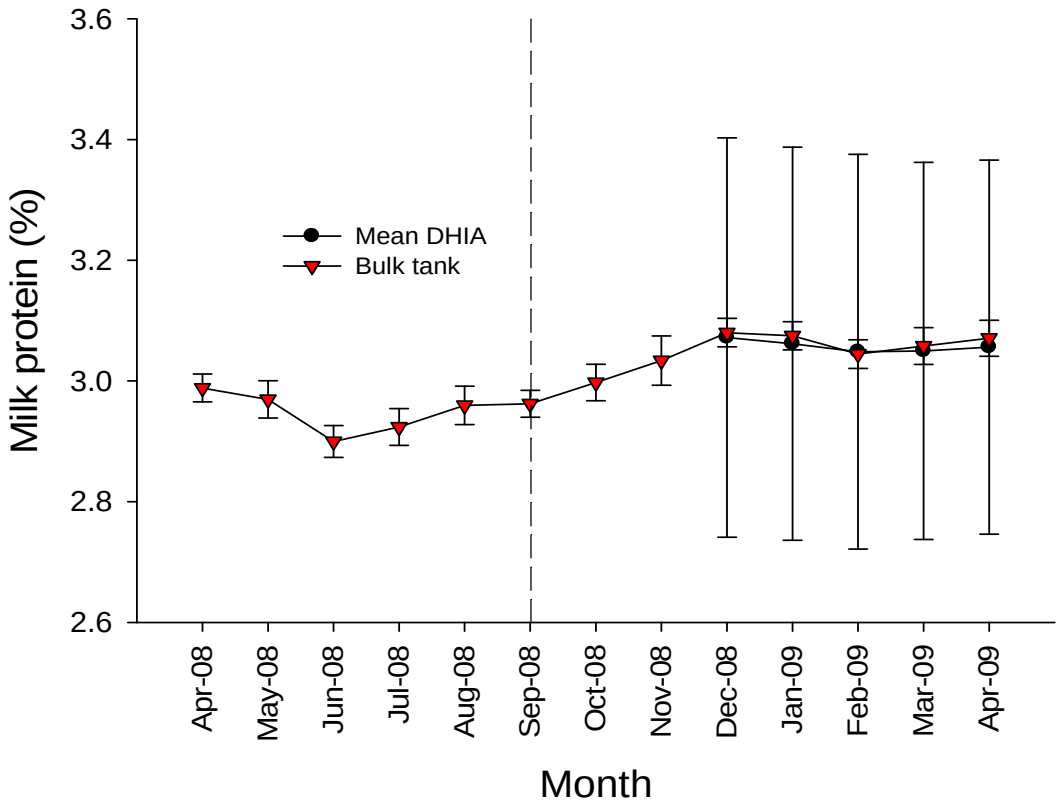

Figure 4.4.12: Mean milk protein from bulk tank (Apr 08-09) and DHIA (Dec-08 to Apr-09) testing. The dashed line represents the start of the study. Bulk tank values represent the whole herd average; mean DHIA represents the weighted mean of pen 1, 2, 3 and 8.

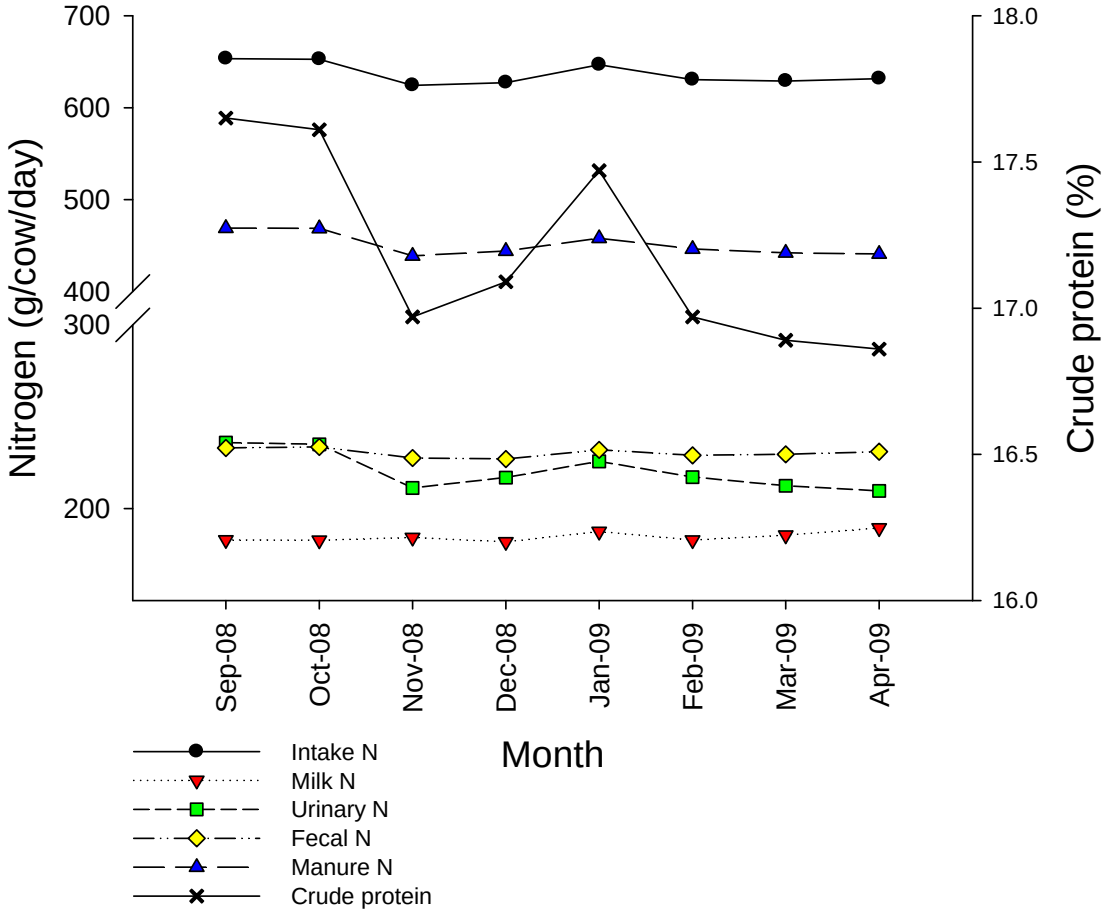

Figure 4.4.13: Mean N balance and ration CP for the average cow in pens 1, 2, 3 and 8 from Sep-08 to Apr-09.

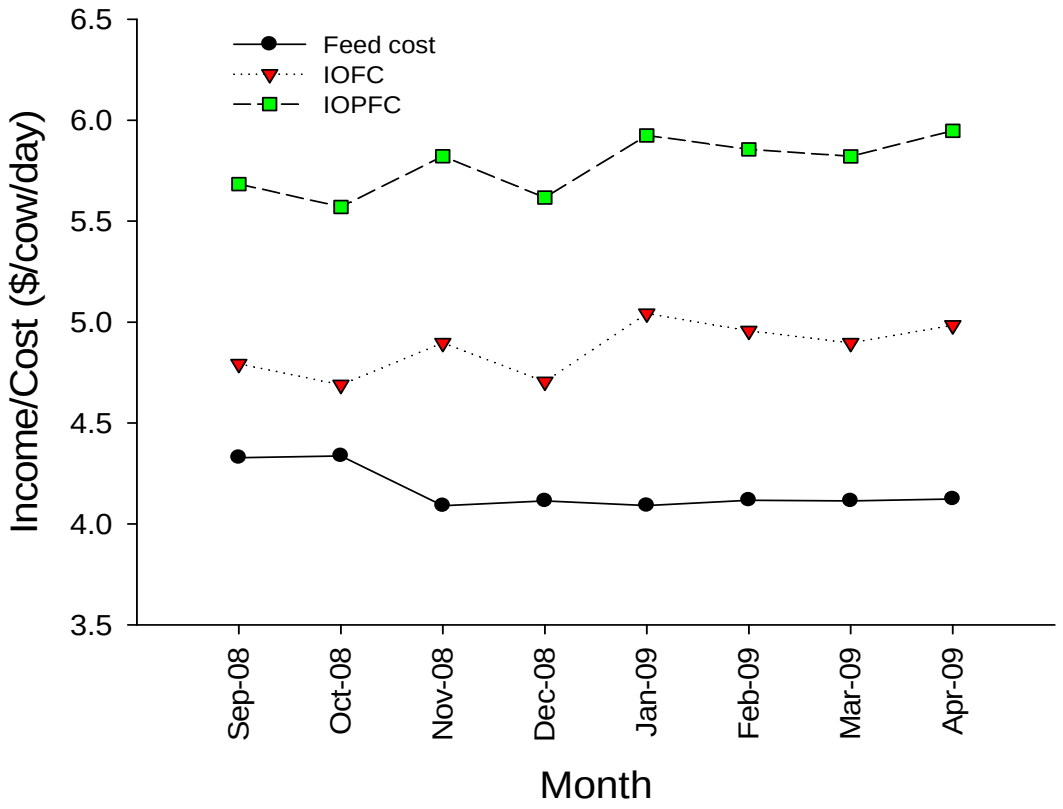

Figure 4.4.14: Feed cost, income over feed cost (IOFC) and income over purchased feed cost (IOPFC) for the average cow in pens 1, 2, 3 and 8 from Sep-08 to Apr-09.

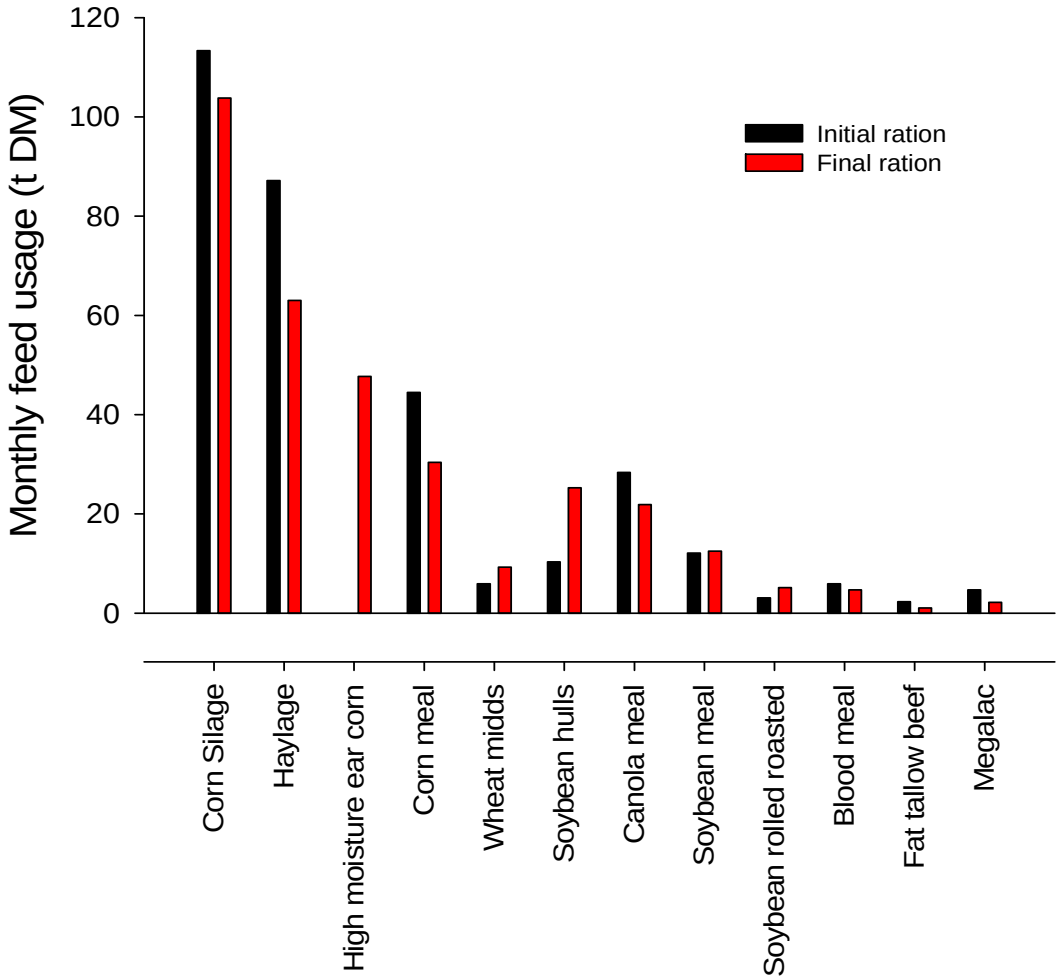

Figure 4.4.15: Difference in the use of specific ration ingredients each month for the milk cows (pens 1, 2, 3 and 8; 450 cows; refer to Figure 4.4.9 for milk production) from the initial ration to the final ration. Ration changes are summarized in Table 4.4.2.

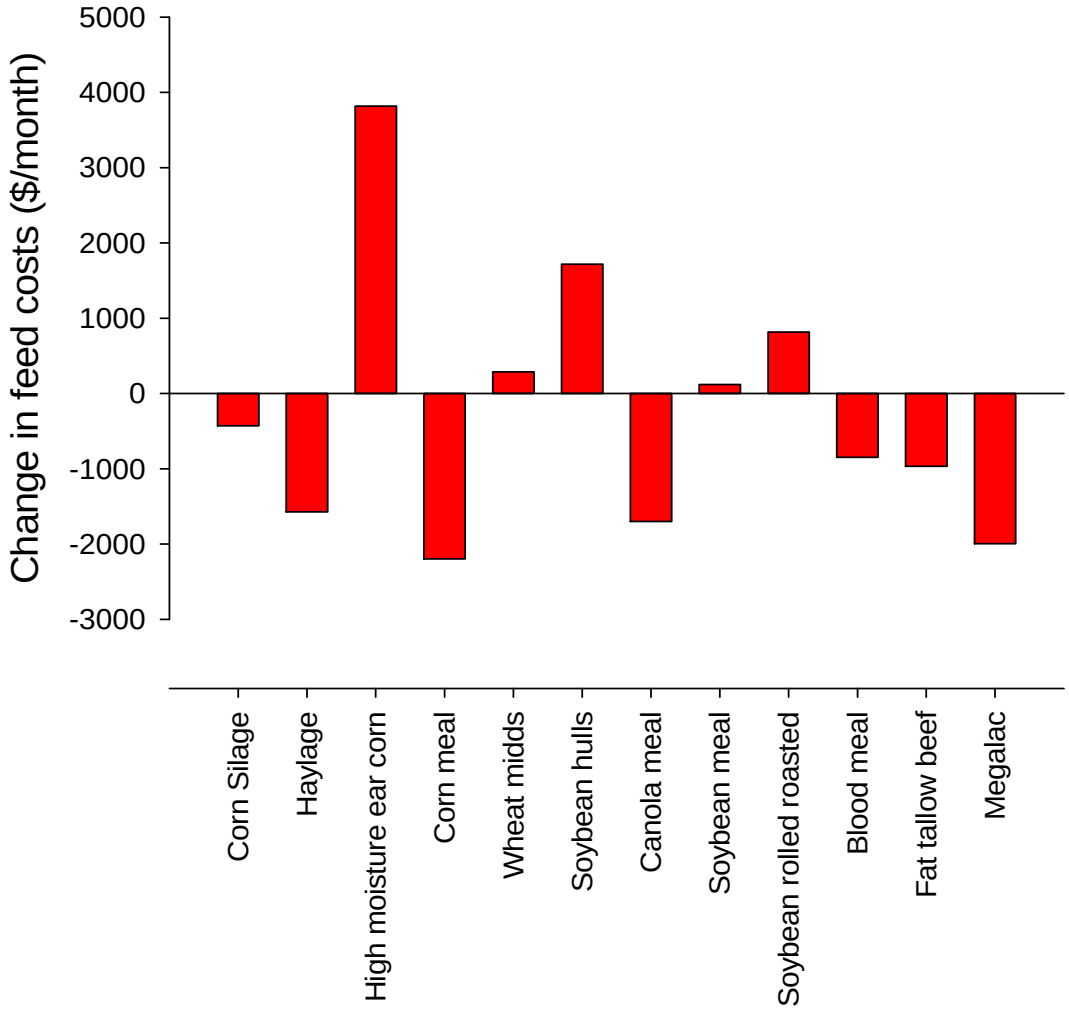

Figure 4.4.16: Difference in the amount spent each month on various ration ingredients for the milk cows (pens 1, 2, 3 and 8; 450 cows) from the initial ration to the final ration.

Table 4.4.2: Ration composition over the trial period for the milk cows (pens 1, 2, 3 and 8) on Farm B.

Ingredient (lbs/cow/day)	**Sep-08**	**Oct-08**[a]	**Nov-08**[b]	**Dec-08**[c]	**Jan-09**[d]	**Feb-09**	**Mar-09**[e]	**Apr-09**
Corn silage	16.25	15.63	13.94	13.63	14.49	13.78	14.80	14.88
Haylage	12.50	10.80	10.17	10.03	10.19	10.23	9.13	9.03
Dry hay	1.70	1.70	1.67	1.65	0.87	0.88	0.88	0.88
High moisture corn	0.00	1.50	4.11	4.09	4.11	4.87	5.83	6.84
Corn meal	6.38	6.16	5.42	5.65	5.69	5.72	5.05	4.36
Sugar	1.10	1.10	1.08	1.07	1.08	1.08	1.09	1.09
Molasses	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Wheat midds	0.85	1.34	1.33	1.34	1.35	1.36	1.34	1.33
Soybean hulls	1.48	2.18	3.13	3.16	3.54	3.55	3.58	3.62
Canola meal	4.07	4.06	4.03	4.07	3.74	3.76	3.45	3.14
Soybean meal	1.74	1.74	1.73	1.74	1.75	1.76	1.77	1.79
Soybean rolled roasted	0.44	0.44	0.44	0.44	0.45	0.45	0.59	0.74
Corn gluten feed dry	0.43	0.43	0.43	0.43	0.43	0.43	0.44	0.45
Corn gluten meal 60%	0.55	0.55	0.55	0.55	0.55	0.56	0.55	0.55
Blood meal	0.85	0.85	0.60	0.60	0.61	0.61	0.64	0.67
Fat tallow beef	0.33	0.30	0.15	0.15	0.15	0.15	0.15	0.15
Megalac	0.67	0.63	0.30	0.30	0.30	0.30	0.31	0.31
Urea	0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Alimet	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Smartamine	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Minerals and vitamins	1.46	1.46	1.48	1.49	1.52	1.52	1.57	1.60
Intake (lbs DM/cow/day)	51.00	51.07	50.70	50.57	51.00	51.19	51.31	51.61

[a] Introduction of a lower digestibility 3rd cutting alfalfa haylage caused a 4lb drop in milk. Changes were made in response to this.
[b] Changed from 07 to 08 Corn silage (Not fully fermented) and the first experimental diet was fed.
[c] Corn silage still not fully fermented.
[d] Haylage changed from 3rd cutting alfalfa to 2nd cutting mixed mostly legume.
[e] Haylage changed from 100% 2nd cutting mixed mostly legume to 50% 2nd cutting mixed mostly legume and 50% 4th cutting alfalfa.

Key Points

Working with commercial farmers provided the opportunity to apply scientific theory to real world situations with all the environmental, economic and human resource constraints of a typical dairying business. Hence, significant care was taken to ensure the changes proposed protected the farm (did not increase costs or reduce income), and the nutritionist (the farm maintained confidence in the nutritional service provided) while still meeting the objectives of the study (see section 4.2). Important changes observed during the study are below:

- Average milk production fluctuated on both farms over the course of the study. Initial and final milk production on Farm A was similar (81 lbs/cow/day). Farm B decreased production by approximately 1 lb/cow/day from the time the first experimental diet was introduced (Nov-08) to the end of the study (Apr-08).

- Milk fat and protein increased on both farms. Farm B maintained the increase throughout the study whereas farm A finished the study approximately where it began.

- Feed costs decreased from $7.47 to $6.30 and $8.49 to $7.99/100 lbs DM for Farms A and B, respectively.

- Income over feed cost increased by $0.99 and $0.16/cow/day for Farms A and B, respectively. This was attributed to a reduction in feed costs, but also an increase in the yields of fat and protein.

- Nitrogen utilization was improved on both farms. The amount of N in the feces and captured in the milk remained constant while the amount of N lost in the urine decreased. Ration CP was reduced by approximately 1% on both farms which

corresponded to a mean decrease in MUN of approximately 2 mg/dl over the course of the study.

The dynamic nature of forage composition and inventory constraints meant that adjustments were continually being made to keep the rations as consistent as possible. Ration CP was reduced as a result of increasing the ratio of corn silage to haylage and replacing protein concentrates (soy bean meal and blood meal) with carbohydrates (corn meal and soy hulls). Literature data would suggest that if amino acids were balanced, CP could still be reduced by up to 1% of DM on both farms at the current level of milk (Armentano et al., 1997; Austin et al., 1991; Broderick, 2003; Castillo et al., 2000; Dinn et al., 1998; Grings et al., 1991). However, a practical limit may have been reached in terms of what the farms and nutritionists were comfortable with in what was a relatively short term study. There were numerous additional challenges that had an impact on the extent to which N utilization could be improved. These included:

- Major forage changes – 2008 corn silage crops were less digestible than 2007 crops and were fed prior to being fully fermented. Hay crop silages were changed numerous times during the study (see Tables 4.4.1 and 4.4.2, respectively) in response to inventory and quality constraints which added additional variability to the diets.

- Milk and feed prices – Milk prices were low and feed prices were high. This meant both businesses were trying to implement cost saving strategies. Farm B was attempting to feed the herd to a 0% refusal to avoid wasting feed which may have restricted intake and potential milk production.

- Bovine somatotropin – Both farms initially used bST on eligible cows. Farm A continued through the entire study. However, Farm B took approximately 70 cows off in Jan-09 due to pressure from the milk processor which may also have influenced potential milk production.

- Cow numbers – Farm A had stable cow numbers over the study period. Farm B increased numbers from 432 (pens 1,2,3 and 8) in Sep-08 to a peak of 480 in Dec-08 and back down to 458 by the end of the study. The peak in cow numbers in Dec-08 corresponded to a 6 lb/cow/day reduction in milk which, subsequently, recovered in Jan-09 when cow numbers decreased to 439. This indicates over-crowding may have caused the drop in milk production.

- Confidence in model predictions – It was evident that there was a lack of confidence in the predictions of both, ruminal-N and total MP supply of CNCPS v6.1 from both the nutritionist and the farmers. This will need to be addressed with additional farm level studies and extension support.

- Consistency – More progress was able to be made on Farm A than Farm B which was partly because the management of the herd was more consistent.

Commercial level studies are challenging due to the financial risk the farm must assume without formal compensation. The economic environment experienced during this study was particularly difficult and forced a more conservative approach than otherwise may have been taken. Despite this, the data confirm that on commercial farms, excess ration CP can be successfully reduced without negatively impacting

milk income, and that updates to the CNCPS enable the development of diets with less environmental impact.

4.4.2 Part 2 – Characterizing commercial herds with high N utilization

The first part of this chapter dealt with improving N utilization in herds with opportunity to reduce N intake. Characterizing herds that are successfully feeding low protein diets can provide additional insight into formulation strategies which can be applied in other situations. In addition, targets for N utilization can be established that demonstrate 'best practice standards'. Basic information on the farms analyzed is summarized in Table 4.4.3. As mentioned previously, the farms included were from three different states (NY, PA and WI), and of different sizes (45-1550 milking cows). Important commonalties among the rations fed and herd performances are:

1. Milk production and protein yield – All but two herds (8 and 11) were producing greater than 80 lbs/cow/day. All herds averaged milk true protein concentrations of 3.00% or above (Table 4.4.3).

2. Forage level and type – Every ration except ration C consisted of 50 to 60% forage (% DM; Figure 4.4.17). Many rations were composed of predominantly corn silage. In cases where higher levels of hay crop were fed, corn grain levels were higher. Farm K was the only farm that did not feed corn silage.

3. Starch and NFC levels – Starch and NFC levels were high in every case (Table 4.4.4). Most rations supplied greater than 28% starch (% DM) and greater than 40% NFC (% DM).

4. Ration NDF content and intake – NDF levels were generally greater than 30% DM and forage NDF as a percent of full body weight (FBW) was generally close to 0.90, indicating that the high energy levels did not displace fiber or forage intake (Table 4.4.4).

5. CP, AA balance, and ruminal ammonia levels – Rumen ammonia concentrations were maintained at levels above requirement in every ration despite the low levels of CP. Crude protein concentrations ranged from 14.3 to 16.5% DM with most rations containing less than 16% CP. When using the CNCPS to evaluate diets, practical target formulation levels for Lys and Met of 6.7-6.8 and 2.2-2.3 % MP, respectively, are suggested (Schwab and Boucher, 2007; Schwab and Ordway, 2004). Most diets were below these recommendations despite achieving high milk and milk protein yields. There may be an opportunity in these herds to improve amino acid balance and improve milk protein yield.

6. MP and ME allowable milk – ME allowable milk was generally higher than MP allowable milk which was due in part to the low dietary protein levels, but also high dietary energy levels. Some herds were predicted to be short on MP supply at the stated level of milk production. Previous versions of the CNCPS (v5) would have predicted MP supply in the current rations to be severely less than required as described by Van Amburgh et al. (2007).

The current data reflect trends seen in the literature, where the relationship between milk and milk protein yield, and dietary energy intake is more important than that seen with dietary protein intake (Broderick, 2003; Griinari et al., 1997). Multiple reasons

have been suggested for this relationship including increases in microbial protein synthesis (Henning et al., 1993; Kim et al., 1999; Sairanen et al., 2005), increases in the supply of glucose precursors that drive milk production (Broderick, 2003), and also a regulatory response from insulin and the IGF system (Bauman, 2000; Griinari et al., 1997). A combination of all three factors is likely. Importantly, the rations in the current analysis supplied high levels of forage and fiber simultaneously with high levels of readily fermentable energy, which would have a positive impact both on the cost of the ration, but also the health of the animal.

Various studies have demonstrated little benefit from supplying in excess of 16% CP (Austin et al., 1991; Broderick, 2003; Grings et al., 1991), particularly when diets are balanced for Lys and Met (Armentano et al., 1997; Dinn et al., 1998). The current data agree with these suggestions and confirm that cows in commercial herds can produce high milk yields on rations under 16% CP. Both Lys and Met were predicted to be below the recommended practical targets in many rations. Rations D, E, F, H and J demonstrate it is possible to supply high levels of Lys even when rations are composed of high levels of corn. Interestingly, the stated rations fed higher levels of Met than the 3:1 ratio of Lys to Met recommended to avoid Met wastage (Schwab and Boucher, 2007; Schwab and Ordway, 2004). Rumen-protected Met supplements were needed to elevate Met to the levels formulated for in the rations, indicating that the herd's nutritionist was probably targeting these higher levels.

Interesting trends in N utilization were observed. Fecal N losses were very constant when presented as a proportion of N intake (Figure 4.4.18). Productive N and urinary N were more variable and correspond almost directly to each other. Productive N is determined by two major factors: milk yield, and milk protein concentration.

Therefore, farms that are able to maximize the proportion of N going to milk protein will subsequently minimize urinary N losses. The proportion of productive N to total intake N was over 35% in many rations and 38% in ration F. Efficiencies as high as 43% (Frank and Swensson, 2002), 37% (Noftsger and St-Pierre, 2003) and 36% (Recktenwald, 2007) have been reported in the literature, but on commercial farms, these efficiencies tend to be much lower. It must be noted that the N efficiencies in the current data are calculated from formulated DMI and may vary from actual intakes. Despite this, MUN concentrations confirm a high level of N utilization (Table 4.4.5). Monthly bulk tank MUN values show that farm 4 has been consistently improving N utilization for the past 6 months whereas farm 7 has maintained a similar level over the same time period (Figure 4.4.19). These data give insight into a number of important points:

1. High levels of N utilization can be maintained over the long term (farm 7).

2. Adjustments targeted to improve N utilization should be completed in small increments rather than an abrupt change (farm 4).

3. High levels of milk and milk protein can be produced when MUN concentrations are 8-10 mg/dl.

Table 4.4.3: Basic details on the farm, diet and milk production from cows being fed rations A-P.

Farm	**1**	**2**	**3**	**4**	**4**	**4**	**5**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Ration	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**
Farm details																
Milking cows	1550	108	270	920	920	920	120	120	100	700	60	180	45	220	45	250
State	WI	WI	WI	NY	NY	NY	NY	NY	WI	NY	NY	PA	PA	PA	PA	PA
Diet																
DMI (lbs/cow/day)[a]	50.0	54.0	53.5	53.0	60.0	52.0	54.5	54.5	60.1	50.5	42.3	55.6	48.0	48.1	49.7	55.1
ME (% required)	99	108	111	109	105	107	111	107	112	107	117	109	111	108	110	116
MP (% required)	99	99	117	105	102	108	100	104	112	107	105	97	99	95	95	105
Cow																
Milk (lbs/cow/day)	88	88	85	83	116	93	89	92	85	89	60	95	80	75	85	85
Milk fat (%)	3.60	3.60	3.80	3.60	3.20	3.40	3.65	3.65	4.00	3.50	4.00	3.60	3.60	3.85	3.70	3.56
Milk true protein (%)	3.05	3.20	3.07	3.40	3.00	3.00	3.00	3.17	3.00	3.10	3.10	3.10	3.10	3.20	3.20	3.03
Milk:feed (%)	1.73	1.62	1.83	1.69	1.99	1.95	1.64	1.77	1.65	1.92	1.53	1.62	1.64	1.45	1.60	1.66

[a] DMI represents the level used by the nutritionist to formulate the ration.

Table 4.4.4: Description of important forage, energy and protein/nitrogen parameters for rations A-P.

Farm	**1**	**2**	**3**	**4**	**4**	**4**	**5**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Ration[a]	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**
Forage																
Forage (% DM)	57.0	60.4	47.7	54.8	60.1	60.1	58.7	57.7	56.9	52.5	50.2	51.2	59.4	51.5	59.3	55.1
Forage NDF (% FBW)	0.88	0.86	0.86	0.86	0.94	0.85	0.99	0.94	0.91	0.88	0.78	0.99	0.89	0.78	0.89	1.02
Forage CP (% DM)	10.9	11.0	15.3	11.3	11.0	10.3	10.0	11.1	14.4	11.5	17.3	12.5	14.7	15.6	17.0	10.6
Forage NDF (% DM)	41.7	36.9	45.9	45.9	40.4	42.1	47.8	44.9	43.6	46.6	47.6	47.0	42.0	42.7	40.9	42.4
CS (% forage DM)	80.4	72.1	36.9	62.0	68.0	72.0	53.1	65.2	46.5	64.0	0.0	58.1	55.9	48.9	37.9	73.6
Energy																
NFC (% DM)	43.4	41.9	40.6	40.6	41.5	40.8	42.4	43.4	38.1	39.1	40.0	39.3	41.3	40.7	44.4	42.5
NDF (% DM)	28.9	30.8	30.7	32.3	30.9	31.6	31.4	30.1	31.5	32.2	30.5	32.3	29.3	31.5	29.3	31.5
Starch (% DM)	28.5	27.1	31.6	29.1	28.7	28.6	29.3	33.8	24.0	27.6	26.3	28.7	28.6	27.6	29.5	28.6
Sugar (% DM)	3.5	3.1	4.2	5.1	5.4	5.2	5.0	3.8	3.3	5.1	7.0	3.5	3.7	3.4	4.1	7.4
EE (% DM)	4.3	3.8	4.3	4.9	5.1	5.5	4.4	4.1	5.2	5.4	5.4	5.1	5.1	4.8	4.0	5.2
Protein/nitrogen																
CP (% DM)	15.9	15.5	16.1	15.9	15.9	15.7	14.3	15.2	16.0	16.3	16.5	15.8	15.6	15.0	15.6	15.5
MP (g/cow/day)	2625	2720	2961	2863	3306	2880	2599	2864	3016	2792	1991	2744	2305	2256	2419	2739
BP (% MP)	47.7	48.3	47.8	45.6	45.9	46.8	51.4	46.5	45.8	45	46.6	47.5	49.0	53.8	55.3	48.8
Lys (% MP)	6.59	6.23	6.39	6.69	6.74	6.82	6.42	6.76	6.17	6.64	5.63	5.77	6.32	6.23	6.31	6.29
Met (% MP)	1.94	1.96	2.05	2.71	2.71	2.69	1.91	2.3	1.77	2.79	1.78	1.85	1.91	1.88	1.91	1.93
Lys:Met (% MP)	3.41	3.18	3.12	2.46	2.49	2.54	3.36	2.94	3.49	2.38	3.16	3.12	3.31	3.32	3.3	3.26
NH_3-N (% required)	137	133	119	125	117	109	140	126	164	123	186	159	152	138	133	142

[a] FBW = Full body weight, CS = Corn silage, NFC = Non-fiber carbohydrates, EE = Ether extract, BP = Bacterial protein.

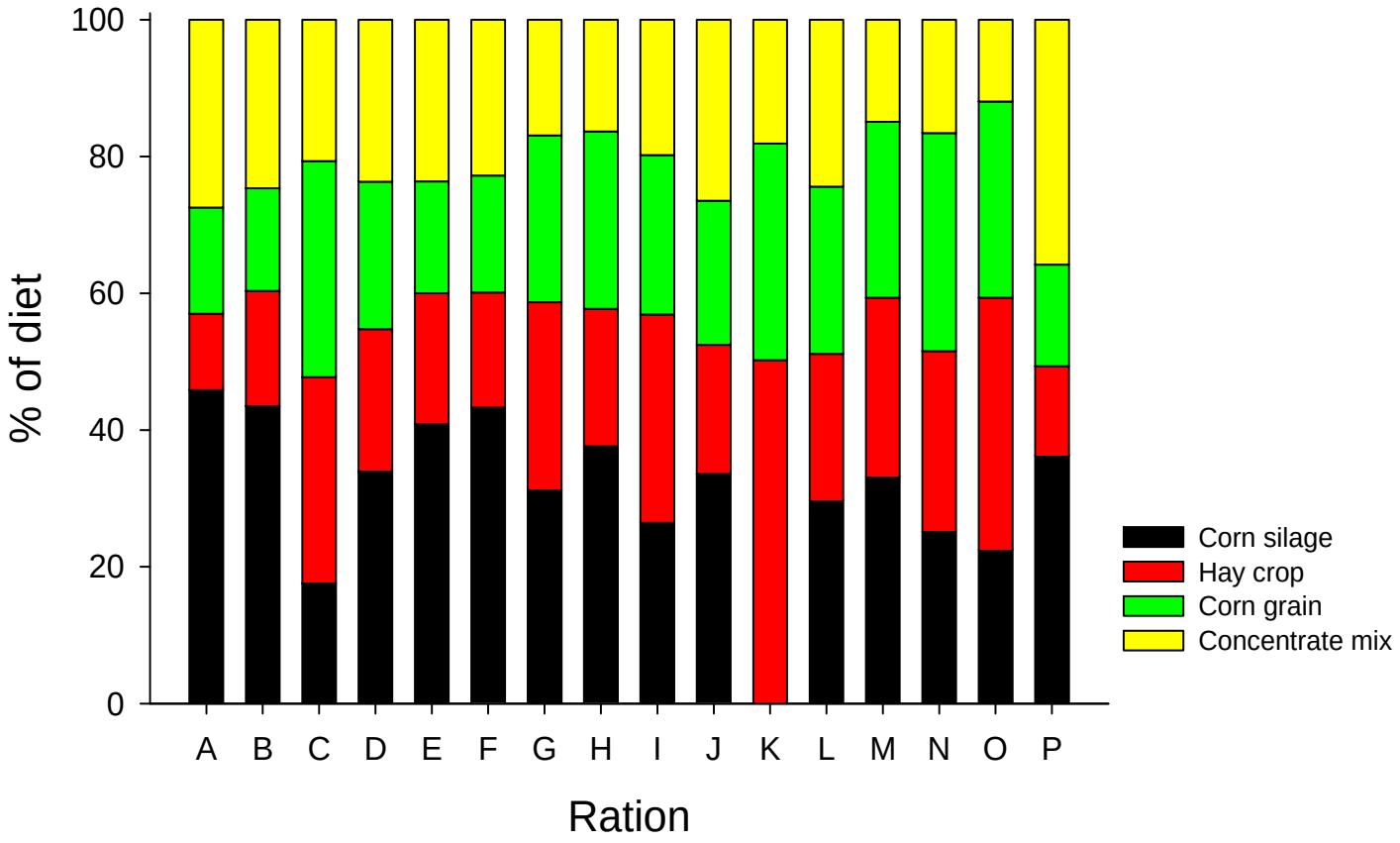

Figure 4.4.17: Diet composition as a percent of the ration for rations A-P (see Table 4.4.4 for additional ration information).

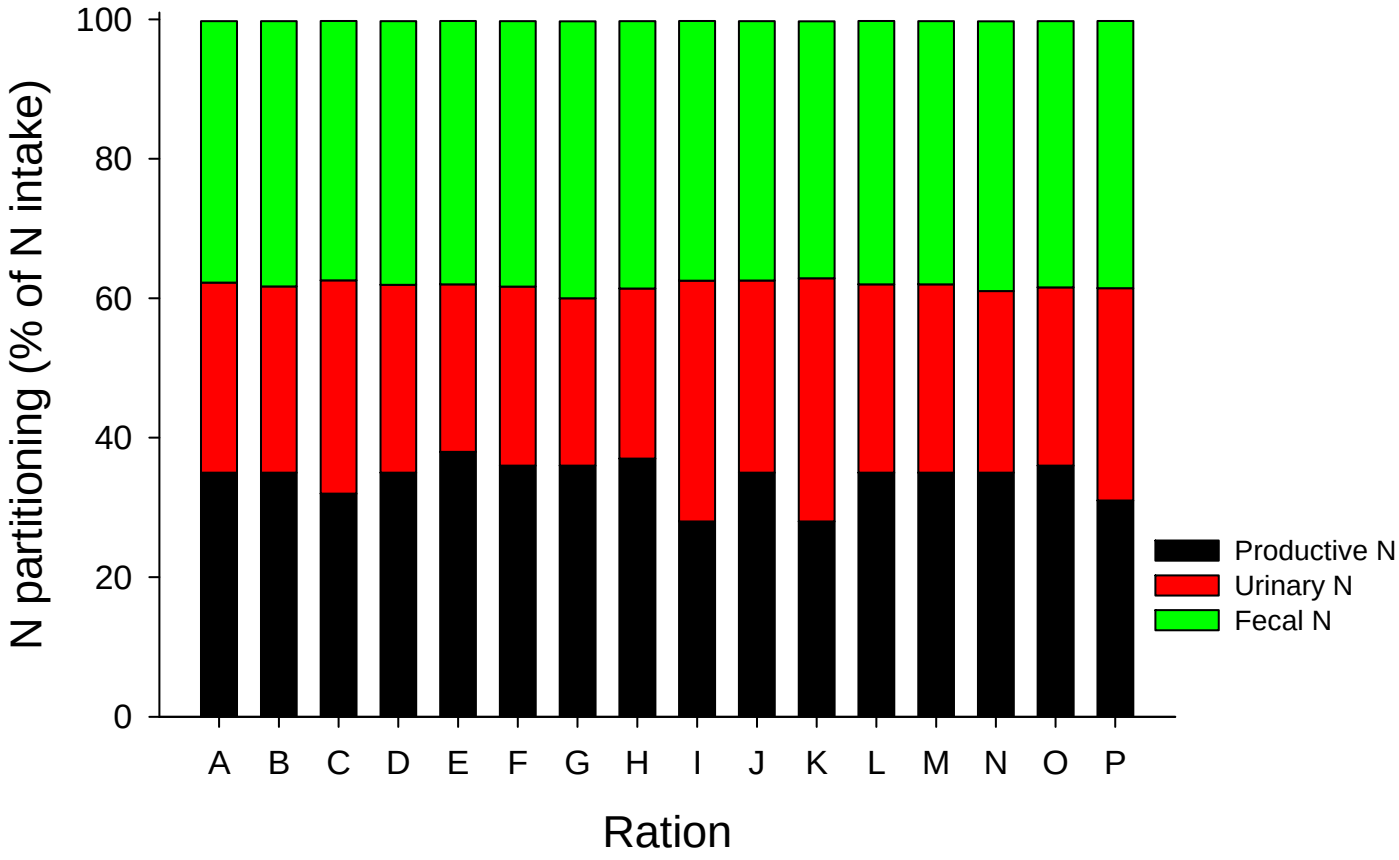

Figure 4.4.18: Nitrogen partitioning in cows fed rations A-P (see Table 4.4.4 for additional ration information and Table 4.4.5 for additional information on N utilization).

Table 4.4.5: Nitrogen partitioning and utilization of cows fed rations A-P.

Farm	**1**	**2**	**3**	**4**	**4**	**4**	**5**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Ration	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**
Intake N (g/day)	579	609	626	609	690	591	566	602	698	596	507	638	542	522	563	620
Productive N (g/day	203	213	200	213	262	213	204	223	195	208	142	223	190	183	203	192
Fecal N (g/day)	217	232	233	230	260	225	225	231	260	222	187	241	204	202	215	238
Urinary N (g/day)	146	150	179	151	152	139	123	134	227	152	165	160	134	124	132	177
Total manure N (g/day)	363	382	412	381	413	363	348	365	487	373	352	401	339	326	347	414
MUN (mg/dl)	10.6	12	n/a	8.1	8.1	8.1	8-10	8-10	9	8.4	9	8-9	8-9	8-9	8-9	10
Productive N/Total N (%)	35	35	32	35	38	36	36	37	28	35	28	35	35	35	36	31
Productive N/Urinary N (g)	1.39	1.42	1.12	1.41	1.72	1.53	1.66	1.66	0.86	1.37	0.86	1.39	1.41	1.47	1.54	1.09
Manure N/Total N (%)	63	63	66	63	60	62	61	61	70	63	69	63	63	62	62	67
Manure N/Productive N (g)	1.79	1.79	2.06	1.79	1.58	1.71	1.71	1.64	2.49	1.79	2.48	1.80	1.79	1.78	1.71	2.15

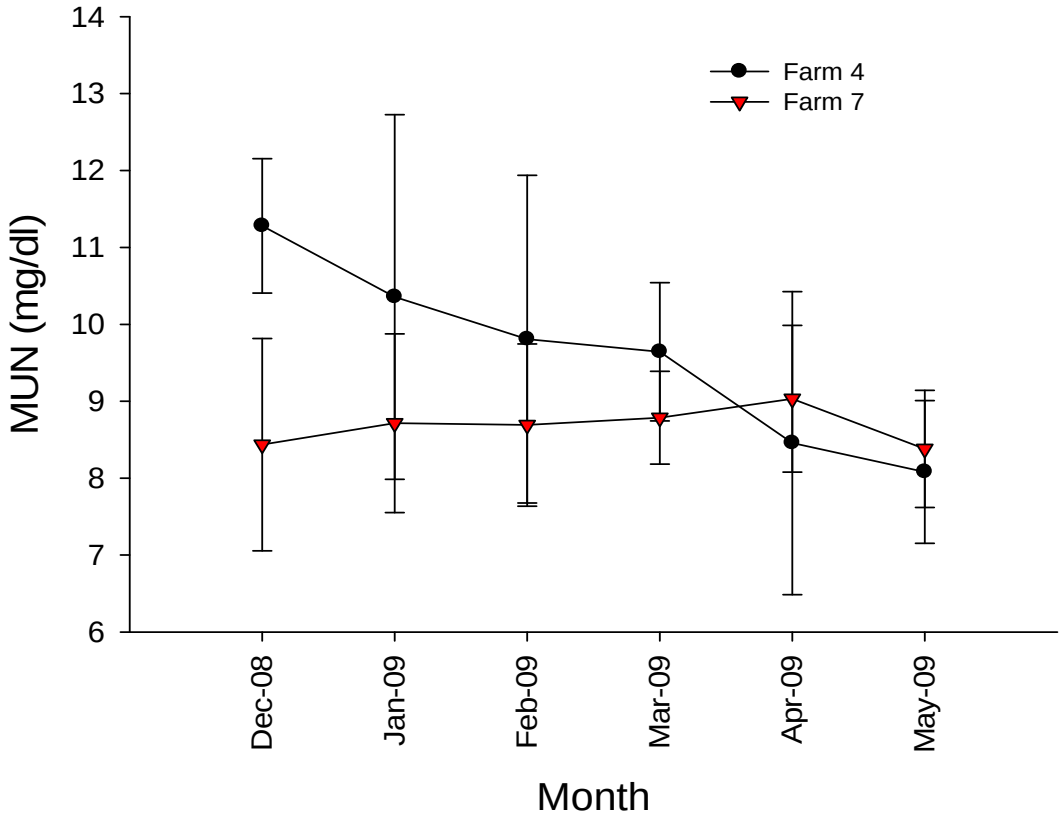

Figure 4.4.19: Whole herd average MUN concentrations from Dec-08 to May-09 for farms 4 and 7.

4.5 Conclusions

Data from part 1 of this study confirm that (1) for commercial herds, ration CP can be successfully reduced without negatively impacting milk income, and (2) updates to the CNCPS enable the development of diets with less N excretion than previous versions. Part 2 showed that commercial herds can achieve high milk yields (>80 lbs/cow/day) on rations consisting of less than 16% CP and as low as 14.3% CP. Key similarities between the herds summarized were:

- High levels of forage (50-60% of ration DM).
- High levels of NDF (30-32% DM).
- High levels of NFC (40-45% DM), and starch (24-33.8% DM).
- Low CP (14.3-16.5% DM).
- Lys and Met levels close to recommendations seen in the literature.

The current data agreed with literature findings. However, the CNCPS predicted that dietary MP was below requirements in some herds. It would be interesting to investigate if the herds predicted to be short of MP supply would benefit from more protein, or does this suggest that the CNCPS is still over-predicting MP requirements?

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