# Ruminal Nitrogen Recycling and Nitrogen Efficiency in Lactating Dairy Cattle

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## ABSTRACT

Excess nitrogen (N) excretion from animal agriculture results in reduced air and water quality, and poses a risk to human health. Although the dairy industry utilizes milk urea N (MUN) to monitor protein feeding and N excretion, phenotypic diversity among cows may influence MUN and thus bias feed management.

An initial study using data from 2 previously published research trials and a field trial, observed that cow had a significant effect on MUN variation. Regression models, utilized to predict MUN, corrected for dietary nutrients and some animal effects, and thus the observed effect of cow on MUN variation may reflect genetic selection decisions of animals with either poor or efficient urea transport.

A second trial observed that MUN and PUN concentrations were positively correlated with gut urea clearance, providing evidence for differences in urea transport activity among cows. The presence of urea transport variation suggests that current protein recommendations may not estimate true requirements.

A third trial observed that animals fed sub-NRC levels of RDP and RUP had reduced N intake and excretion of fecal N, urinary urea-N, and MUN. Animals maximized N efficiency and had no loss in milk production, suggesting a possible overestimation of RDP and RUP in the current NRC prediction model.

The present project provides evidence for phenotypic variation among cows, which may be partially explained by differences in urea transport activity. Future work confirming genetic variation among urea transporters may provide an opportunity to improve feeding management if cow urea efficiency is known.

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## ATTRIBUTION

Several colleagues aided in the writing and research behind Chapter 2 of this thesis project. A brief description of their contributions is included here.

**Chapter 2:** Cow and herd variation in milk urea nitrogen concentrations in lactating dairy cattle.

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# CHAPTER 1: Literature Review

## 1.1 Introduction

Nutrient runoff from animal feeding operations (AFO) has come under the scrutiny of public perception and government agencies. The United States Environmental Protection Agency (USEPA) has identified animal agriculture as a major contributor of nitrogen (N) pollution to water resources and ammonia emissions to the atmosphere (Thomann et al., 1994, USEPA, 2004). The application of manure to crops or livestock excretion of urinary and fecal N, results in nutrient run off, leaching or volatilization. Excessive N inputs into the ground or emitted into the air cause reduced water and air quality, which pose significant human health risks (James et al., 1999, USEPA, 2004). Consequently, current agricultural research has been faced with the challenge to minimize livestock N excretion while maintaining production.

The rumen requires nitrogenous substrates, such as ruminally degradable protein (RDP), for microbial growth and the production of microbial protein (Huntington and Archibeque, 2000, Parker et al., 1995, Russell et al., 1992). Microbial protein and dietary sources of protein, such as ruminally undegradable protein (RUP), contribute to the pool of available metabolizable protein (MP) utilized by dairy cattle to meet amino acid requirements (Satter and Roffler, 1975). After enzymatic digestion of MP and absorption across the small intestine, amino acids are synthesized into proteins to meet maintenance, growth, reproduction, and lactation requirements (Webb, 1990). Therefore, lactating dairy cattle rations must be formulated to provide sufficient N for rumen microbe and animal functions, while minimizing excretion of excess in urine or feces.

Ruminal microbes also obtain N via the recycling of urea from blood to the digestive tract (Huntington and Archibeque, 2000, Lapierre and Lobley, 2001, Parker et al., 1995, Reynolds and Kristensen, 2008). Excess dietary protein and ruminal ammonia are converted into urea within the liver, and released into blood (Parker et al., 1995). On average 33% of synthesized urea is excreted in urine and 67% is recycled to the gastrointestinal tract (Lapierre and Lobley, 2001). Thus, as urea synthesis is driven by protein catabolism the production, recycling, and excretion of urea must be proportional to the intake of dietary N.

Synthesized urea is released into blood and equilibrates with bodily fluids including milk (Broderick and Clayton, 1997, Huntington and Archibeque, 2000). As a result, blood urea N (BUN) and milk urea N (MUN) are highly correlated with dietary N intake (Nousiainen et al., 2004). Additionally, since kidney urea clearance functions as a concentration gradient, urinary N excretion is also proportional to dietary N intake, BUN, and MUN (Jonker et al., 1998). The routine measurement of MUN by milk processors and dairy herd improvement (DHI) testing laboratories has led to the use of MUN as a tool to monitor protein intake and consequently potential N emissions on dairy operations (Jonker et al., 1998, NRCS, 2011).

Strategies to minimize N excretion include reduction of fed dietary N and improvement of N efficiency via dietary management and breeding programs (Lapierre and Lobley, 2001, Reynolds and Kristensen, 2008). Studies that decreased dietary CP below NRC requirement have observed reduced of ammonia emissions from dairy cows and their manure (Broderick, 2003, Colmenero and Broderick, 2006b, Reynal and Broderick, 2005). Also the individual reductions of dietary RDP or RUP below NRC (2001) recommendations was able to meet RDP and RUP needs respectively, as

evidenced by the lack of affect affecting milk production (Agle et al., 2010, Cyriac et al., 2008, Rius et al., 2010). The results of these studies suggest an over-estimation of RDP and RUP in the current NRC (2001) protein model. Current inadequacies of the NRC protein model may be the result of insufficient data or the treatment of urea recycling as a constant in prediction equations. Therefore, the role and influence urea recycling plays in true protein requirements must be determined.

Several factors can cause deviations in expected MUN values (Broderick and Clayton, 1997, DePeters and Cant, 1992, Kauffman and St-Pierre, 2001). Genetic differences between cows must be present as MUN is a heritable trait (Miglior et al., 2007, Mitchell et al., 2005, Stoop et al., 2007, Wood et al., 2003). Given that urea synthesis is driven by protein catabolism, it is unlikely that urea synthesis could be the source of MUN variation if animals are consuming similar amounts of feed and producing similar amounts of milk protein. However, variation in urea transport activity in the kidney and rumen wall could be the source of observed animal differences (Marini et al., 2004, Marini and Van Amburgh, 2003). For example, if animals have poor urea transport into rumen or kidney MUN concentrations will be high and animals may be more susceptible to RDP deficiencies in the rumen, which may compromise microbial growth. Thus animal variation in urea transport activity, reflected in MUN concentration, may be a driving determinant of true RDP requirements for lactating dairy cattle.

**1.2 Nitrogen Emissions from Agriculture**

Nonpoint source (NPS) pollution is the accumulation of natural and human-made pollutants in ground water and their eventual runoff into lakes, rivers, wetlands, coastal waters, and underground sources of drinking water (USEPA, 1977). The Chesapeake Bay Program has identified that excess nutrients (USEPA, 1983), specifically NPS

pollution (Thomann et al., 1994), as the primary reason for the water quality decline observed in the Chesapeake. Angle et al. (1986) estimated that NPS pollution contributed approximately 67% of the N that reached the Chesapeake Bay. Dairy operations are a large agricultural enterprise in this region and the mismanagement of dairy manure is major a contributor of N loading to the Chesapeake Bay (Shirmohammadi et al., 1997).

Excess N fed to lactating dairy cattle results in significant losses of N in either feces or urine. On average livestock excretion of feces and urine contains only trace amounts of inorganic N (ammonia, nitrites, and nitrates) while the majority consists of organic N (urea and undigested protein) (USEPA, 2004). Once exposed to the environment organic N can go through the process of ammonification and nitrification, and thus large organic N losses from densely stocked regions of dairy cattle can result in high levels of nitrate in groundwater and the eutrophication of surface water (Shirmohammadi et al., 1997). The overloading of nutrients, such as N, into bodies of water increases the growth of algae and aquatic weeds (Carpenter et al., 1998). As a result, increased senescence and decomposition of algae and aquatic weeds causes oxygen shortages and kills fish (Carpenter et al., 1998). The consequences of eutrophication include the loss of marine habitats, reduced marine biodiversity, and pose a potential risk to human health (Anderson, 1994, NRC, 1993, Seehausen et al., 1997). Algal blooms, such as red or brown tides, release toxins that have severe negative impacts on aquaculture, cause shellfish poisoning in humans, and significantly increase mortality in marine mammals (Anderson, 1994, Shumway, 1990).

Alternatively, N excreted in dairy cattle urine and feces can also be volatilized resulting in reduced air quality. Approximately 1 to 5% of fecal N is immediately

volatilized to ammonia (Lockyer and Whitehead, 1990), while stored feces slowly release ammonia year-round (Patni and Jui, 1991). In contrast, urinary N is excreted as organic N, or urea, and rapidly hydrolyzed into two moles of ammonia by urease enzymes present in the soil, plant roots, and animal feces (Elzing and Monteny, 1997). The newly formed ammonia either volatilizes or stays in solution where it forms ammonium carbonate (Anderson et al., 2003). Excessive ammonia present in the atmosphere reacts with oxides of N and sulfur (S) forming particles less than 2.5 microns in size ($PM_{2.5}$), which if deposited within the lungs may lead to increased morbidity or mortality (Anderson et al., 2003). The negative environmental impacts and potential human health risks associated with dairy cattle excretion provides an incentive for dairy producers to minimize N losses to groundwater and the atmosphere.

The amount of excreted N that is volatilized from livestock manure depends on several factors, including the N content of the diet, animal size, breed, housing, humidity, temperature, concentration, pH, and animal waste handling practices (Anderson et al., 2003, USEPA, 2004). Large animal production facilities have limited land space, resulting in densely stocked animals and excess waste production (Anderson et al., 2003). Dairy cattle are fed diets higher in N as compared to beef cattle, and as a result a dietary management difference in N excretion has been observed (Acker and Cunningham, 1998). As the pH of stored livestock manure is typically around 7.0, increased climate humidity and temperature promote increased chemical and soil microbe reactions, thus enhancing the potential for excreted N to be volatilized and emitted into the air (USEPA, 2004). Thus various other factors, such as stocking density, production system, and environmental conditions, must be considered when evaluating the true extent of

environmental damage and impact caused by livestock, and more specifically dairy cattle, nutrient excretion.

Within the United States, levels of N and S oxides in the atmosphere have dropped 50 to 80% since 1980 respectively, due to regulations set forth in the National Ambient Air Quality Standards (NAAQS) (USEPA, 2012). Sources of N and S oxides include emissions from automobiles, power plants, large industries, and agriculture (USEPA, 2012). Current EPA secondary standards were created to protect against environmental damage caused by oxides of N and S (USEPA, 2012). The most recent secondary standard established for N oxides was set at 0.053 ppm averaged over a year. Additionally, the United States Clean Air Act (CAA) states under section 10, that it is the responsibility of individual states to implement a plan to identifying sources of agricultural pollution and determine strategies of pollution reduction to meet federal air quality standards (USEPA, 1963).

The United States Clean Water Act (CWA) developed a concentrated animal feeding operation (CAFO) strategy to reduce the negative impact of nutrient run off (USEPA, 1977). Under this program the EPA utilized national enforcement incentives to identify and improve problems existing with CAFOs (USEPA, 1977). Additionally, the more recently released Clean Water Action Plan (CWAP) requires the U.S. Department of Agriculture (USDA) and the U.S. Environmental Protection Agency (EPA) to develop a Unified National Strategy to minimize the water quality and public health impacts of CAFOs (USDA and USEPA, 1998). The Unified National Strategy is based on a comprehensive nutrient management plan (CNMP) with nutrient management goals of improved feed management, manure handling and storage, land application of manure, land management, record keeping, and other utilization options that all CAFO owners

and operators are expected to implement and follow. Thus, the United States is continuously working toward agricultural pollution prevention and preservation of environmental health. Furthermore, the EU enacted Council Directive 91/676/EEC as a strategy to protect bodies of waters against agricultural sources of N pollution (EU, 1991). The active progress of both the EU and the United States toward reducing agricultural pollution and preserving the environment reflects the importance of developing more effective strategies to reduce dairy N excretion.

### 1.3 Nitrogen Efficiency of Dairy Cattle

Lactating dairy cattle have relatively low N efficiency as they transfer 25 to 30% of consumed N to milk or tissue N, while the remainder is excreted in urine and feces (Bequette et al., 2003, Castillo et al., 2000, Wilkerson et al., 1997). Gastrointestinal infusion of protein or amino acids (AA) and dietary supplementation of protein can increase milk protein content and yield, but observed responses have been unpredictable and lower than expected values (Bequette et al., 1998). However, observed increases in milk protein due to protein supplementation, can result in a decrease of N efficiency (Broderick, 2003, Ipharraguerre and Clark, 2005). Thus, improvements in lactating dairy cattle N efficiency are necessary to increase N capture and reduce N losses to the environment (Jonker et al., 2002a).

Nitrogen losses occur across the gastrointestinal tract (GIT), liver, and mammary gland (Hanigan, 2005, Lapierre et al., 2006). The magnitude of N losses across these tissues is variable (Hanigan, 2005), and there is debate over which tissues are the primary cause of poor AA efficiency post-absorption. Lapierre et al. (2006) observed that liver and portal-drained viscera were the principal cause of poor AA efficiency, whereas Bequette et al. (1998) observed that largest loss of branched chain amino acids (BCAA)

occurred across the mammary gland. Splanchnic tissues exhibit a low affinity for AA (Reynolds, 2006), but since splanchnic tissues receive 50% of cardiac output (Davis et al., 1988) they ultimately remove a large proportion of circulating AA. Consequently, the recycling of unused AA to splanchnic tissues results in a 60% loss of available AA on a daily basis (Hanigan, 2005), suggesting that a large proportion of AA are not utilized by peripheral tissues and are then returned to the splanchnic tissues (Hanigan, 2005). Therefore, a better understanding of mammary utilization of AA will reduce recycling, improve post-absorptive AA efficiency, and increase overall N efficiency of the animal.

The uptake of AA by the mammary gland is dependent on arterial concentrations of AA, the rate of mammary blood flow (MBF), and transfer of blood AA across basal membranes of the secretory cells (Mepham, 1982). The arterial concentration of AA and rate of MBF determine the quantity of AA that reach the mammary gland per unit time (Mepham, 1982). Given that the supply of AA is a function of postabsorptive entry, the uptake of AA must be dependent on the affinity of the udder for AA (Bequette et al., 2000, Hanigan et al., 1998b). Under conditions of a single essential AA (EAA) deficiency in the diet, the mammary gland increased AA transport activity for that single EAA by 43-fold and increased MBF by 33% (Bequette et al., 2000). In contrast, the infusion of an AA mixture lacking one EAA caused a decrease in the plasma concentration for that single EAA, which was suggested to be the result of increased uptake (Weekes et al., 2006). The results of these studies indicate that the mammary gland is capable of altering its AA transport activity in response to arterial supply. Thus, variation in postabsorptive AA supply will greatly influence mammary gland uptake, utilization and efficiency.

An incomplete understanding and mathematical model of AA metabolism during and after absorption may explain the unpredictable and low expected values of milk protein response observed in lactating dairy cattle (Armentano, 1994). The current NRC (2001) model assumes the efficiency of use of MP for lactation to be a fixed constant of 0.67. Given that MP supplemented above AA requirements is converted into urea within the liver (Parker et al., 1995) and that urea recycling increases N available for anabolic use post-absorptively (Lapierre and Lobley, 2001), variation in urea transport may directly influence the availability and potentially the efficiency of AA utilization post-absorptively. Thus, future NRC models must account for the dynamic metabolism of AA post-absorptively and the affects of urea transport efficiency in order to predict responses in milk protein output more accurately.

The interrelationship between dietary energy and protein supply also influences N efficiency of lactating dairy cattle (Rotz et al., 1999). Dietary protein provides RDP for rumen microbial protein synthesis (Russell et al., 1992) and RUP, which is directly absorbed and utilized by cows for anabolic purposes (Satter and Roffler, 1975). Dietary energy stimulates rumen microbial protein synthesis, resulting in increased demand for ruminal RDP as well as more available MP for milk protein production (Cadorniga and Satter, 1993). However, rations formulated for maximal milk production must take care not to overfeed dietary protein or energy as they are costly ingredients and excess dietary intake may result in increased N excretion (Broderick, 2003). Rius et al. (2010) observed that post-absorptive energy and CP supply had independent effects on milk production and that maximal N efficiency was achieved when feeding the combination of high energy and low CP(%) in diets fed to lactating dairy cattle. In contrast, prior studies showed no change in N efficiency when cows were fed diets below N requirements

(Hanigan et al., 1998a) or an increase in N efficiency when cows were fed diets low in degradable starch (%) (Castillo et al., 2001). The inconsistent responses of animal N efficiency to dietary energy and protein content also pose another variable that must be considered in future research.

Structural carbohydrates are essential to maintain the health and normal function of the rumen (NRC, 2001). An adequate supply of effective fiber is necessary for proper rumen function and to stimulate chewing (Beauchemin and Yang, 2005). Time spent chewing is dependent on particle size of the forage and is directly correlated to the flow of salivary buffers that neutralize rumen pH (Bailey and Balch, 1961). Rumen buffering and a fiber mat are required to maintain rumen pH above 6.0, which will ensure growth and activity of rumen microbes (Ishler et al., 1996). Diets providing reduced forage particle size will result in decreased retention time, increased rumen turnover rate, and increased rate of passage allowing microbes less time to digest feeds (Ishler et al., 1996). Effective fiber is essential for rumen microbes to adequately degrade dietary RDP and energy in order to efficiently produce microbial protein. Thus, dietary fiber content also plays an important role in a cow's ability to efficiently utilize dietary N.

**1.4 Milk Urea Nitrogen as a Tool to Monitor Feeding Management**

The fate of RDP within the rumen is dependent on various factors to ensure maximal microbial growth, microbial protein production, ruminal digestion, and nutrient availability to the animal without excessive ammonia production (Reynal and Broderick, 2005). Additionally, microbial protein plays an important role in milk production as it provides half to two thirds of the MP utilized by lactating dairy cattle (Council, 1992, Ishler et al., 1996). Diets containing excess RDP, above NRC requirements, will induce the production of large amounts of ammonia in the rumen (Colmenero and Broderick,

2006a). Ruminal ammonia is absorbed into blood, transported to the liver and converted into urea (Huntington and Archibeque, 2000). Urea released from the liver equilibrates with body fluids, including milk, resulting in a high correlation between BUN and MUN (Huntington and Archibeque, 2000). Given that BUN diffuses into milk, it is expected that observed MUN levels would be highly correlated with BUN, urea synthesis, RDP and RUP content of the diet (Nousiainen et al., 2004, Schepers and Meijer, 1998). Thus, due to the routine measurement of MUN values, dairy producers can use MUN concentrations to monitor dietary RDP content.

Proper dietary content of RUP and utilization by lactating dairy cows can also be monitored using MUN. Dietary RUP is undegraded by rumen microbes and passes directly into the GIT where it is enzymatically digested and released AA are absorbed into portal circulation (Webb, 1990). Dietary RUP fed above nutrient requirements can cause excess circulation of free AA, leading to increased recycling of N to the GIT (Hanigan, 2005). Excessive rumen microbe utilization and conversion of recycled free amino acids (FAA) into ammonia, in addition to FAA circulating in blood, will cause an increase in urea synthesis and therefore in MUN concentration (Reynal and Broderick, 2005). Thus dietary RUP content, which results in excess circulating AA and urea concentrations, can also be monitored through MUN concentration to reduce overfeeding and ensure proper MP requirements are supplied to the animals.

Excess energy supply in the diet also poses a problem to feed management as dietary energy stimulates rumen microbe activity and results in increased microbial protein synthesis, which can contribute to excess MP converted to urea by the liver (Broderick, 1998). Excessive production of microbial protein will also increase microbial catabolism of AA to ammonia and will cause a build up of rumen ammonia

concentration (Broderick, 1998). Higher ruminal ammonia concentration increases liver urea synthesis, release to blood, and equilibration with body fluids such as milk. Therefore MUN concentration can be used to monitor dietary RDP, RUP, and energy as a strategy to reduce the feed cost and excess intake of dietary protein and energy.

The goal of dairy protein nutrition is to provide sufficient dietary protein via minimal N intake with maximal N utilization into milk protein without compromising yield. A large reduction in N intake that does not affect or improves milk yield results in animals with increased N efficiency (NRC, 2001). An ideal dairy total mixed ration (TMR) adequately supplies rumen microbes with energy, RDP, and fiber and animal tissues with energy and AA without surpassing true requirements (Broderick, 2003). The balance created from appropriate quantities of these dietary nutrients will result in minimal ammonia production, AA deamination, and urea synthesis (Lobley et al., 1995). Under these conditions the mammary gland can maximize its absorption and utilization of AA for milk protein synthesis. Milk urea levels may therefore reflect dietary N intake, rumen microbe activity, and urea synthesis. Thus, MUN may also be useful in monitoring animal N efficiency if N intake and milk N yield are known.

Many animals, including ruminants, have evolved the capability to synthesize urea as a mechanism to remove excess N as a method to prevent the toxic build up of ammonia (Huntington and Archibeque, 2000). On average, once released into blood, 67% of urea synthesized enters the GIT while the remaining 33% is eliminated in urine (Lapierre and Lobley, 2001). Of the 67% that enters the GIT 50% is reabsorbed as AA, 40% is reabsorbed as ammonia, and 10% is lost in feces (Lapierre and Lobley, 2001). The fraction of urea eliminated in urine is first filtered through the nephrons of the kidneys and removed from blood as a result of a concentration gradient created by

counter current flow and differences in membrane permeability of the ascending and descending loops of Henle (Swenson and Reece, 1993). MUN has been observed to be highly correlated to urinary N excretion (Jonker et al., 1998). Urea recycling within dairy cattle may influence the fraction of urea eliminated in urine, and thus MUN can also be used as a tool to monitor the excretion of N into the environment.

Another concern regarding excess N in dairy cattle is the negative impact that urea has on reproductive performance. Traditional selection of dairy cattle for improved milk production traits has resulted in an undesirable decline in cow health and reproductive performance (Rogers et al., 1999). Studies have observed that urea has a toxic effect on sperm and ova (Dasgupta et al., 1971, Umezaki and Fordney-Settlage, 1975), and can cause abortion when injected intra-amniotically (Greenhalf and Diggory, 1971). A study performed by Jordan et al. (1983) observed that ova and sperm viability was reduced when animals were fed diets with excessive protein content. Additionally, studies have reported that dairy cows with high BUN concentrations had reduced conception rates (Elrod and Butler, 1993, Ferguson et al., 1988), suggesting that high BUN levels exacerbate the severity of negative energy balance post-parturition. Rhoads and colleagues observed that high plasma urea N (PUN) concentrations in lactating dairy cows decrease embryo viability through effects exerted on the oocyte 7 days after insemination (Rhoads et al., 2006). Thus, MUN values can potentially be a monitoring tool for reproductive improvement in lactating dairy cattle.

The negative genetic correlation between milk production traits and cow health has led to increased efforts to develop selection criteria that will improve animal health. Several studies have observed genetic variation amongst cows for disease resistance, but heritability estimates for these traits were generally low (Lin et al., 1989, Simianer et al.,

1991, Van Dorp et al., 1998). Given that MUN is a heritable milk production trait (Wood et al., 2003) and is routinely measured by DHI programs, suggests that MUN may have phenotypic relationship with common diseases that afflict dairy cattle. A review written by Ingvartsen et al. (2003) describes the use of indicators, such as serum urea N, for early disease prevention as tools on a modern dairy farm. However a recent study performed by Mitchell et al. (2005) observed no significant relationships between estimated breeding values for MUN and diseases. The lack of repeatable evidence supporting a correlation between MUN and cow health indicates that further investigation into this field of research is needed.

Given that the amount of urea excreted in urine by a cow is directly proportional to BUN and MUN, studies have examined the use of MUN to predict urinary N excretion. Ciszuk and Gebregziabher (1994) observed that MUN and urinary N excretion had correlations of 0.64 and 0.73 amongst goats and dairy cattle respectively, indicating that MUN should be a good predictor of urinary N excretion. Mathematical models have since been developed that utilize MUN values to estimate urinary N excretion by lactating dairy cattle. Jonker et al. (1998) developed the earliest set of prediction models that integrated N intake, fecal N excretion, and milk N output. Data from 3 digestibility and N balance studies used to develop this model estimated a MUN target range of 10 to 16 mg/dl to minimize N excretion without affecting milk production (Jonker et al., 1998). Kauffman and St-Pierre (2001) have enhanced this prediction model by incorporating the significant influence of body weight (BW) into the equation. Thus, MUN has proven to be a useful tool in predicting the harmful excretion of N into the environment.

Ample evidence has demonstrated that routine MUN measurements can be used by dairy operations to reduce excess nutrient intake, improve N efficiency, improve

reproductive performance, and ultimately reduce N excretion through the use of prediction models. Implementation of MUN as a monitoring tool on dairy farms was investigated by Jonker et al. (2002b). It was hypothesized that providing dairy farmers in the Chesapeake Bay Drainage Basin with information regarding herd MUN would result in more accurate feed management and MUN levels closer to target MUN values. Results indicated that 89.5% of dairy farmers did not routinely use MUN prior to participating in the project although 88% of the extension agents and nutritionist in the region recommended the use of MUN to balance rations. By the end of this project 30% of farmers responded that they would use MUN analysis in the future to monitor feeding practices. Thus the results of this study indicate that providing information regarding the usefulness of MUN monitoring can change feeding management on dairy operations.

**1.5 Variation in Milk Urea Nitrogen**

Although MUN can be used as a good indicator of proper feeding management, several factors are known to cause variation in MUN concentration. Excess N production as a result of high DMI, deviations in forage protein, improperly balanced rations, or improperly mixed rations can contribute to variation in MUN. Reduced water intake, or dehydration, will result in increased BUN and therefore MUN (Steiger Burgos et al., 2001). Kauffman and St-Pierre (2001) observed that BW had a significant impact on the variation associated with MUN concentration, prompting them to include BW as a factor in predicting urinary N excretion. A review written by DePeters and Cant (1992) describes variation in total milk N content due to environmental temperature, disease, stage of lactation, parity, breed, and dietary nutrient content. Additionally Wattiaux et al. (2005) observed a significant effect of breed on MUN variation, which was dependent on

whether a cow belonged to a single-breed herd or a multiple-breed herd indicating that there may be a substantial effects from individual cows.

The effect of cow on MUN variation may be partially explained by the observed heritability of MUN amongst lactating dairy cattle. Heritability estimates for MUN amongst Holstein cattle in lactations one, two, and three were 0.44, 0.59, and 0.48 respectively from field data collected by dairy herd improvement programs (Wood et al., 2003). Heritable variation for MUN amongst Holsteins across all lactations was 0.15 and 0.22, despite the two separate analysis performed on milk samples collected (Mitchell et al., 2005). Additionally, average daily heritabilities for MUN amongst Canadian Holstein ranged from 0.383 to 0.414 (Miglior et al., 2007). The presence of MUN heritability amongst lactating dairy cattle supports the influence of genetic variation on MUN differences observed.

Given the heritability of MUN, it is possible that sire selection decisions within a herd may have resulted in herds and animals with intrinsically high MUN concentrations. The high estimates for MUN heritability also indicate that selection of future animals based on MUN is possible and could result with cows and herds with improved urea recycling efficiency. Bouwman et al. (2010) conducted a study to detect quantitative trait loci (QTL) affecting MUN concentration in an effort to understand the underlying genetic variation observed in MUN. The goal of QTL studies is to find genetic markers that can be implemented into breeding programs through marker assisted selection (Khatkar et al., 2004). Animals included in the QTL study were genotyped for 1,341 single nucleotide polymorphisms (SNP) and 4 chromosomal regions were identified with suggestive QTL (Bouwman et al., 2010). The authors of this study concluded that QTL affecting MUN concentration and yield were suggestive and each explained 2 to 3% of observed

phenotypic variance (Bouwman et al., 2010). Thus, the identification of suggestive QTL indicates that further exploration into genetic differences between lactating dairy cattle is necessary to fully understand true N requirements.

Urea transporters within the kidney (You et al., 1993) and rumen wall (Ritzhaupt et al., 1997, Stewart et al., 2005) were recently discovered and could be the source of animal variation observed for MUN. Additionally, more than 1 allele for the urea transporter gene has been identified (Marini et al., 2004, Marini and Van Amburgh, 2003, You et al., 1993). Currently the genomic structure of only one urea transporter has been determined in bovine rumen epithelium, and is known as UT-B (Stewart et al., 2005). Isozaki et al. (Isozaki et al., 1994) observed that under conditions of protein restriction, net urea flux across rat kidneys was significantly increased ($P < 0.02$). The authors suggest that two urea transporters may have been involved in the adaptation observed when animals were fed diets low in protein. The results of this study were the first to elucidate the role of urea transporters under conditions of varying dietary protein content.

The identification of urea transporters and their potential role in N recycling led to the work of Marini and Van Amburgh (2003), which demonstrated that protein intake may induce differential abundance of urea transporters in the ruminal mucosa of Holstein heifers. To further investigate the role of urea transporters, Marini et al. (2004) performed a trial to determine the relationship between GIT, liver, and kidney tissue extraction of urea with N recycling in lambs fed diets varying in protein content. When fed diets varying in CP content, urea transporter abundance in the kidney and GIT tissue did not reflect urea absorption by the kidney or urea transferred to the GIT. The results of this study indicate that urea transporter abundance may not be the source of MUN

variation observed among lambs, which may also include other ruminants such as dairy cattle.

The recent advancement of veno-arterial difference techniques has allowed various research groups to investigate true nutrient absorption across splanchnic tissues. The veno-arterial difference approach involves anatomical placement of permanent indwelling catheters in various veins and arteries of a research animal (Lapierre and Lobley, 2001). This technique can be performed in both steady-state or non-steady-state conditions and allows research scientists to separate liver and gut metabolism (Lapierre and Lobley, 2001). Rojen et al. (2011) utilized this technique to determine the relationship between BUN concentration, GIT urea extraction, and urea transporter expression in cows supplied with decreasing RDP levels. The reduction of dietary RDP content fed to lactating cows did not result in increased urea recycling to the GIT. Instead, Rojen et al. (2011) observed that the reduced N supply led to higher urea extraction across rumen and portal drained viscera (PDV) tissue. However, no correlation between UT-B abundance mRNA and changes in N supply were observed. Thus, the results of this study further support that urea transporter abundance is not the source of variation causing differences in BUN and MUN between lactating dairy cattle.

Differences in urea transport activity may be the source of animal variation for MUN, as no relationship was found between urea transporter abundance and urea N recycling. Cows with poor urea transport would be expected to have high MUN concentrations and may be susceptible to ruminal N deficiencies, whereas cows with efficient urea transport will be the opposite. If urea transport is poor, depressed microbial growth, microbial protein production, and MP available for animal utilization may occur.

Thus, if animal variation in urea transport is present, MUN values can be used as a feed management tool to group animals based on their efficiency to transport urea.

The most recent target MUN concentration was established at 12 mg/dl or less (Simpson et al., 2009). This target MUN concentration is thought to indicate proper protein feeding of lactating dairy cows, which will result in minimized N loss postabsorptively and N excretion via feces and urine (Simpson et al., 2009). Current NRC and urine prediction models were utilized to set this target, and therefore it is believed that this particular MUN concentration will not affect milk production (Simpson et al., 2009).

Various phenotypic factors that effect MUN concentrations pose a problem to dairy producers that want to maintain herds at or below target MUN values. Environmental factors, such as humidity or temperature, may cause herd MUN to increase past target MUN values despite farmer compliance with protein feeding recommendations (DePeters and Cant, 1992). Additionally, potential genetic variance amongst animals may also predispose a herd to abnormally high MUN levels. A static regulatory target for MUN concentration may not work for herds with intrinsically high MUN levels and may result in lost milk production if microbial growth and protein production are compromised. Animals with poor urea transport in either the rumen or kidney will have low blood urea transport to body fluids such as urine or milk, resulting in increased BUN. Although these animals have reduced urea transport, the increased concentration of BUN will overcome poor urea transport, resulting in similar urea excretion as compared to cows with normal urea transport. Thus, if urea transport differences amongst cows are not accounted for in current NRC and excretion models, producers may be penalized for failing to meet target MUN levels even though cows are

not consuming or excreting more N as compared to herds with intrinsically low MUN levels.

If urea transport differences exist amongst cows, the current target MUN value must be improved to account for this variation. Herd MUN concentrations will need to be calibrated for individual herds based on efficiency of urea transport in order to avoid biased feeding decisions. Assessment of herd feeding programs and accounting for all environmental and genetic factors must be performed when performing calibrations for a herd to achieve a realistic target MUN concentration. Thus, the potential existence and influence of variation in urea transport activity must be considered in future regulatory discussions regarding target MUN and feed management programs.

**1.6 Research Objectives and Hypotheses**

The importance of reducing the environmental footprint of commercial dairy operations has become an essential need as global pollution worsens. Strategies to manipulate dietary protein intake have been the most common method to reduce N excretion from dairy cattle. Given the possible presence of urea transport variation amongst dairy cattle and the use of MUN as an indicator of N efficiency, the present project worked toward elucidating a relationship between urea transport and MUN concentration. Thus the first research objective was to determine variation associated with animal and herd MUN levels, while accounting for differences in dietary nutrient content. It was hypothesized that cow variation in MUN could affect overall herd MUN and bias feed management decisions.

The specific source of variation associated with animal MUN level is necessary to fully understanding true N requirement and N recycling within lactating dairy cattle. The cause of variation observed in MUN can be affected by various factors such as DMI,

water intake, breed, or BW. Despite prediction models accounting for these various factors, variation in MUN is still observed. Thus the second objective was to determine the relationship between MUN and urea transport into the GIT. It was hypothesized that cows with low MUN concentrations would have high rates of urea transport into the rumen as compared to cows with high MUN concentrations.

Currently, urea recycling is included as a constant among cows in prediction equations and the current NRC model for protein requirement. As urea return to the GIT provides a source of RDP, it can be assumed that the current NRC model for protein requirement will over-estimate true requirement for RDP. Urea recycling to the rumen may supply a sufficient amount of RDP to maintain microbial activity and production of microbial protein. Maintenance of microbial activity, via urea recycling, may also result in sufficient MP to maintain animal health and production. However, variation in urea transport activity may be the source of poor transport activity among cows, thus resulting in animals with higher BUN and MUN but lower transfer of urea to the GIT and in urine. On the other hand, RDP and RUP concentrations could be adjusted to avoid compromising rumen microbial activity, since microbes require RDP and a portion of urea recycled to the rumen arises from RUP. Thus the third objective was to determine the interactions of varying dietary RDP and RUP concentrations on milk production, microbial protein synthesis, and animal N efficiency. It was hypothesized that dairy cattle may be able to maintain performance when fed a combination of sub-NRC requirement levels of RUP and RDP.

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**CHAPTER 2: Cow and Herd Variation in Milk Urea Nitrogen Concentrations in Lactating Dairy Cattle**

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## ABSTRACT

Milk urea nitrogen (MUN) is correlated with N balance, N intake, and dietary N content, and thus is a good indicator of proper feeding management with respect to protein. It is commonly used to monitor feeding programs to achieve environmental goals; however, there is also genetic diversity among cows. It was hypothesized that phenotypic diversity among cows could bias feed management decisions when monitoring tools do not consider genetic diversity associated with MUN. The objective of the work was to evaluate the effect of cow and herd variation on MUN. Data from 2 previously published research trials and a field trial were subjected to multivariate regression analyses using a mixed model. Analyses of the research trial data showed that

MUN concentrations could be predicted equally well from diet composition, milk yield and milk components regardless of whether dry matter intake was included in the regression model. This indicated that cow and herd variation could be accurately estimated from field trial data where feed intake was not known. Milk urea N was correlated with dietary protein and NDF content, milk yield, milk protein content, and days in milk for both data sets. Cow was a highly significant determinant of MUN regardless of the data set used, and herd trended to significance for the field trial data. All other variables being held constant, a percentage unit change in dietary protein concentration resulted in a 1.1 mg/dl change in MUN. Least squares means estimates of MUN concentrations across herds ranged from a low of 13.6 mg/dl to a high of 17.3 mg/dl. If the observed MUN for the high herd was caused solely by high CP feeding, then the herd would have to reduce dietary protein to a concentration of 12.8% of DM to achieve a MUN concentration of 12 mg/dl, likely resulting in lost milk production. If the observed phenotypic variation is due to genetic differences among cows, genetic choices could result in herds that exceed target values for MUN when adhering to best management practices, which is consistent with the trend for differences in MUN among herds.

Key words: milk urea nitrogen, feeding management, dairy cattle

## INTRODUCTION

Nitrogen emissions from agriculture cause air and water quality impairment (Tamminga, 1992, Van Horn et al., 1996). Animal agriculture has been identified as a major contributor of nitrogen (N) pollution to water resources in the Chesapeake Bay

watershed (Boesch et al., 2001, Fisher and Oppenheimer, 1991), and research resources have focused on identifying management practices to reduce environmental impact (NRC, 2003).

Dairy cattle are commonly fed diets with protein levels exceeding 16% to ensure maximum milk output (NRC, 2001). This practice contributes to the relatively low N efficiency of lactating dairy cattle (Bequette et al., 2003, Huhtanen and Hristov, 2009) while adding dietary cost and potentially decreasing profit margins (Godden et al., 2001). Milk urea nitrogen is highly correlated with urinary N excretion (Jonker et al., 1998, Kauffman and St-Pierre, 2001), and is a good indicator of ammonia emissions from dairy manure (Burgos et al., 2007).

Dietary crude protein available for microbial use in the rumen can be degraded to amino acids and peptides. These are utilized by microbes for microbial protein synthesis, or deaminated and used to support energy needs (see Tamminga, 1979). Provision of ruminally available N in excess of microbial needs for protein synthesis results in generation of ammonia that is absorbed and converted to urea in the liver (Parker et al., 1995). Absorbed amino acids and peptides provided in excess of animal requirements are also deaminated and converted to urea. Thus urea synthesis is proportional to the balance of dietary N and use of N for productive purposes.

Synthesized urea is released into blood and equilibrates with body fluids including milk (Archibeque, 2000, Broderick and Clayton, 1997). This results in high correlations of blood urea N and MUN with dietary N (Nousiainen et al., 2004, Preston et al., 1965). Because the kidney urea clearance is concentration dependent, there is also a high correlation between MUN and urinary N excretion (Jonker et al., 1998). These relationships and routine measurement of MUN by milk processors and DHIA testing

laboratories has led to the use of MUN as a tool for monitoring feeding programs and feed management practices (NRCS, 2011).

Although MUN concentration is clearly related to N balance within a cow, there are several factors that can cause deviations from expected values. These include time of sampling, season of the year, body weight, breed, and nutritional factors (Broderick and Clayton, 1997, DePeters and Cant, 1992, Kauffman and St-Pierre, 2001). There are also significant cow effects (Wattiaux et al., 2005) that are at least partially explained by genetic variance (Miglior et al., 2007, Mitchell et al., 2005, Stoop et al., 2007, Wood et al., 2003). Given the genetic effects on MUN, it is possible that sire selection decisions within a herd may result in herd concentrations of MUN differing from the expected values based on feed management. When the model of Kauffman and St-Pierre (2001) was used to predict MUN concentrations for individual cows in trials performed by Cyriac et al. (2008) and Rius et al. (2010), the variance in residual MUN associated with cow was 4.1 ± 1.1 mg/dl ($P<0.001$), indicating that the cow itself was an important determinant of MUN. Because differences in DM intake were accommodated in the model, DMI could be ruled out as a contributor to the observed cow variance. The model did not exhibit mean bias, although there was a large range in residual errors (mean residual = -0.1 ± 6.5 mg/dl). If herd-level deviations are as large, it could result in poor feed management decisions when using MUN to guide feeding choices.

Studies examining genetic parameters for cow and herd variation in MUN have not utilized dietary information (Miglior et al., 2007, Mitchell et al., 2005, Stoop et al., 2007, Wood et al., 2003). Part of the cow and herd variance, therefore, may be associated with feeding multiple diets within a herd and differing diets across herds (Jonker et al., 1998, Kauffman and St-Pierre, 2001). Conversely, studies examining the

relationships among dietary nutrients and MUN have not reported cow and herd variance. The objective of this study was to determine variation associated with animal and herd MUN levels, while accounting for differences in dietary nutrient content, level of production, and stage of lactation. It was hypothesized that cow variation in MUN could affect overall herd MUN and bias feed management decisions.

## MATERIALS AND METHODS

Intake, dietary nutrients, and production data from two previously published trials (Cyriac et al., 2008, Rius et al., 2010) were used to assess whether MUN could be predicted as well from dietary nutrient concentrations as from nutrient intakes. A total of 68 multiparous Holstein and 12 multiparous Jersey x Holstein crossbreds were included in the data set.

The first trial included observations from 40 mid-lactation cows randomly assigned to 1 of 4 diets that contained 11.3, 10.1, 8.8, or 7.6% RDP (DM basis) with corresponding reductions in dietary CP (Cyriac et al., 2008). The second trial included observations from 40 mid-lactation cows assigned to 1 of 4 diets that contained high (HE, 1.55 Mcal NEl/kg) or low dietary energy (LE, 1.44 Mcal NEl/kg) and high (HP, 6.6% RUP) or low ruminally undegraded protein (LP, 4.6% RUP) arranged in a 2 by 2 factorial design (Rius et al., 2010). Feed samples were analyzed for total N (Perkin-Elmer 2410 Series II, Perkin-Elmer, Norwalk, CT), ether extract (AOAC, 1996); method 920.39), ash (AOAC, 1996); method 942.05), acid detergent fiber (AOAC, 1997), neutral detergent fiber (Van Soest et al., 1991), lignin (AOAC, 1997), soluble CP (Licitra, 1996), neutral detergent insoluble CP (Licitra, 1996), acid detergent insoluble CP (Licitra, 1996), starch

(YSI 2700 Select Biochemistry Analyzer, Yellow Springs, OH), minerals (AOAC, 1997), and gross energy (bomb calorimetry, model 1271, Parr Instruments, Moline, IL). A summary of the data is provided in Table 2. 1.

A second analysis was performed using data from a field trial with 5 herds (predominantly Holstein) that were being intensively monitored for phosphorus feeding plus the Virginia Tech herd (Holstein, Jersey, and various crossbreeds of Holstein, Jersey, Brown Swiss, and Swedish Red). All herds used DHIA testing services, and each herd was feeding a single lactating cow ration. On 2 consecutive test dates, milk production was recorded and milk samples were analyzed for true protein, fat, and lactose by infrared analyses (Fossomatic 4000 Combi infrared analyzer, Eden Prairie, MN) and for MUN using a modification of the Berthelot procedure (ChemSpec 150 Analyzer; Bentley Instruments, Chaska, MN; Dairy Herd Improvement Association, Blacksburg, VA). In total, data were collected from 741 cows.

Samples of the total mixed ration were collected for the 5 herds on the phosphorus project, and ingredients used in the ration were sampled at the Virginia Tech herd. All samples were submitted to Cumberland Valley Analytical Services (Hagerstown, MD) for analyses of total N, NDF, ADF, and ash. Nitrogen was analyzed according to method 990.03 (AOAC, 2000), while NDF, ADF, and ash were analyzed using methods noted previously. Dietary $NE_L$ was predicted from the other nutrients. Dietary nutrient concentrations were calculated for the Virginia Tech herd based on ingredient analyses and dietary inclusion rates. A summary of the data is provided in Table 2. 2.

Regression analyses were performed using the Mixed procedure of SAS (Version 9.2, SAS Institute Inc., Cary, NC). Two models were used for analyses of the research trial data. The first model regressed MUN concentrations on intakes of CP, NDF, ADF,

and starch (DM basis), DIM, milk yield, milk composition, milk component yield, SCC, period, experiment, and BW, and all 2-way interactions of these terms. The second model regressed MUN concentrations on dietary concentrations of CP, NDF, ADF, and starch, DIM, milk yield, milk composition, milk component yield, SCC, period, experiment, and BW, and all 2-way interactions. Variables with *P*-values greater than 0.1, were sequentially excluded from models using a backward elimination procedure. Cow was included as a random variable and effect of cow was tested using a covtest statement.

Field trial data were analyzed in the same manner using the Mixed procedure of SAS (Version 9.2, SAS Institute Inc., Cary, NC). Milk urea nitrogen was regressed on dietary concentrations of CP, ADF and NDF (% of DM), dietary $NE_L$ (mcal/kg DM), DIM, milk yield (kg/d), milk protein (%), and all 2-way interactions. Herd and cow nested within herd were included as random variables in the model. The effects of cow and herd were tested using a covtest statement.

## RESULTS

Results from analyses of the research trial data using models 1 and 2 are presented in Table 2. 3 and Table 2. 4, respectively. When nutrient intakes were included in the regression model, MUN was correlated with intakes of ADF, CP, and starch, interactions of ADF, CP, NDF, and starch intakes, milk yield, milk protein yield, SCC, interactions of ADF intake with milk lactose yield, milk fat yield, and DIM, interactions of CP intake with milk lactose yield and DIM, interaction of NDF intake and milk fat yield, and interactions of starch intake and milk lactose yield, milk fat yield, and DIM (Table 2. 3).

When dietary nutrient concentrations, but not intakes, were included in the model, MUN was highly correlated with milk protein yield, milk lactose yield, milk fat yield, SCC, and the interactions of CP, NDF, starch, and ADF (% of DM), with milk protein yield, milk lactose yield, milk fat yield, and DIM (Table 2. 4). The effect of BW was not significant in either model. Inclusion of nutrient intakes in the model explained slightly more variation, but the Akaike Information Criterion (AIC; Version 9.2, SAS Institute Inc., Cary, NC) only increased from 750 to 758 suggesting that either approach resulted in similar precision. This is likely due to the high correlation between milk yield and energy intake (Brown et al., 1977, Buttazzoni and Mao, 1989, NRC, 2001). Cow was highly significant in both models.

Having established that MUN can be predicted with comparable precision when nutrient intakes are not known, we used model 2 to analyze the larger field data set (Table 2. 5). As previously observed (Nousiainen et al., 2004) and consistent with the research trial analyses, MUN was highly correlated with dietary CP content. An increase of 1 percentage unit in dietary CP resulted in an increase in MUN of 1.04 and 1.24 mg/dl for milk yields of 40 and 30 kg/d, when other parameters in the model where held constant at the mean observed values listed in Table 2. 2. This slope was less than the 1.7 mg/dl observed by Nousiainen et al. (2004). Milk urea N was also associated with dietary NDF, milk yield, and milk protein content and the interactions of dietary CP with milk yield and milk protein, dietary NDF with DIM and milk protein, and milk yield and milk protein. Cow was highly significant as observed for the research data, and there was a trend for a herd effect ($P<.08$).

## DISCUSSION

Urea excretion is ultimately determined by the balance of N intake and N deposited in body and milk protein. Nitrogen intake in excess of deposition is converted to urea by the liver and released into blood. Because BUN concentrations are reflective of urea production by the liver, BUN and MUN are indicative of protein balance and thus useful for dietary protein management (Broderick and Clayton, 1997, Oltner and Wiktorsson, 1983). A variety of factors are known to contribute to excess urea N production, a key being excessive protein or N consumption. Excessive protein consumption can be caused by greater than expected DMI, deviations in forage protein, an improperly balanced ration, and improper mixing of the ration. Inadequate fermentable or total energy in the diet could fail to support the energy needs of the cow causing lower than expected milk production. Dehydration will also result in increased BUN and MUN (Steiger Burgos et al., 2001, Weeth and Lesperance, 1965). Other factors that affect MUN but likely have little effect on urea N excretion include days in milk and breed (Broderick and Clayton, 1997, Kauffman and St-Pierre, 2001). Although BW has previously been observed to affect MUN, we did not observe a similar relationship herein (Broderick and Clayton, 1997, Oltner et al., 1985).

Milk urea nitrogen levels in the research trial data were highly correlated to dietary nutrients, production characteristics, and stage of lactation regardless of whether DMI was considered in the model. Precision analysis of both models indicates that inclusion of nutrient intake explains only slightly more variation in MUN than simply considering dietary nutrient concentrations supporting the management practice of adjusting dietary protein concentrations to achieve changes in MUN. It also supports the use of data from herds with unknown DMI to assess herd variation in MUN.

Based on the results from the field trial (Figure 2. 1), the average herd with cows producing between 30 and 40 kg milk/d would have MUN concentrations below 12 mg/dl only if diets contained less than 16% CP. However, the effect of herd trended to significance (P<. 08). Least squares means for MUN by herd ranged from a low of 13.6 mg/dl to a high of 17.3 mg/dl. Based on an estimated slope of 1.1 mg/dl per unit of dietary CP (Figure 2. 1), the herd with the highest MUN would have to reduce dietary CP approximately 4.8 percentage units to achieve an MUN of 12 mg/dl, if all other factors are held constant. Based on the average observed dietary CP for that herd, the diet would need to be 12.8% CP. It seems unlikely that such a low level of CP could be fed without compromising milk production (NRC, 2001). Of course, other management factors not considered in the regression analyses may also explain such a deviation from the norm, and thus one should assess all aspects of the operation before resorting to reduced protein feeding to achieve an MUN goal.

The significant effect of cow in the research and field trials is clear evidence of phenotypic differences in MUN concentrations among cows that are not explained by N intake, milk yield, BW, or other production related factors. Mitchell et al. (2005) observed that MUN concentrations were heritable, thus there is a genetic component to the observed variation. Urea transporters exist in a number of tissues including the kidney (Yang et al., 2002) and rumen wall (Stewart et al., 2005) and there is more than 1 allele for the gene (Marini et al., 2004, Marini and Van Amburgh, 2003, You et al., 1993). Genetic variation in these transporters could result in variable transport activity that may explain the observed variation in MUN. If an allele codes for a transporter with reduced activity, this would result in elevated concentrations, and the reverse for alleles that code for increased transport activity.

Phenotypic effects on MUN concentrations are potentially problematic from a regulatory standpoint. Environmental or genetic variance may predispose a herd to abnormally high MUN levels even when the herd is following protein feeding guidelines. Attempting to reduce MUN levels to achieve a static regulatory MUN target in such a herd may result in lost production. If MUN is elevated due to low blood urea transport to urine, these cows will be excreting no more urinary N than cows with more active urea transporters when fed the same diet. Such variation would not be accommodated in current excretion models (Jonker et al., 1998, Kauffman and St-Pierre, 2001), and producers may be penalized for failing to meet target levels even though their cows are not excreting any more N than herds with lower MUN. In the absence of selection measures, a producer may have inadvertently selected for a herd of cows that are predisposed to elevated MUN concentrations. Therefore target values for MUN should not be used across herds without calibration for each herd unless some accommodation for normal herd variation is considered. If a common target value is to be used across herds, a safety margin should be included to accommodate those herds with intrinsically high MUN. Variation in MUN for herds that participated in the field trial was 1.6 ± 1.1 mg/dl. Based on this estimate, the target MUN level should be set 1.6 units above the population mean from the prediction models to accommodate intrinsic MUN variation. There should ensure that 83% of well managed herds can achieve the goal if well managed regardless of their genetic selections. However, the estimate of herd variance was calculated from only 6 herds, which limits the precision of the value as evidenced by the large standard deviation.

Herd calibration could be achieved through an assessment of the herds feeding program, taking into account all possible factors that may affect observed variation in

MUN. If the herd is well managed and feeding a balanced diet that does not exceed NRC (2001) requirements for protein and has adequate energy, the prevailing MUN could serve as a calibrated target value for that herd. If the herd is overfeeding protein relative to energy supply and milk production, the ration would have to be rebalanced and fed for a period of 2 or 3 weeks before reassessing MUN. The MUN value achieved after this period of feeding to requirements should reflect the calibrated target for the herd. Having determined a herd target value, deviations in MUN above the target would be indicative of overfeeding protein while deviations below the target MUN may indicate that cows are being underfed protein. In the absence of such calibration, some accommodation for variation associated with cow should be considered when setting guidelines for acceptable MUN values.

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Figure 2. 1 Least squares mean estimates for MUN versus dietary CP predicted from the model summarized in Table 2. 5 with varying milk yield and the observed mean inputs for milk protein, dietary NDF, and days in milk set to values listed in Table 2. 2. ♦=30 kg milk/d, ■=32 kg milk/d, ▲=34 kg milk/d, x=36 kg milk/d, *=38 kg milk/d, ●=40 kg milk/d, solid line=40 kg milk/d regression (y=1.04 CP – 3.0), dashed line = 30 kg milk/d regression (y=1.23 CP – 7.34).

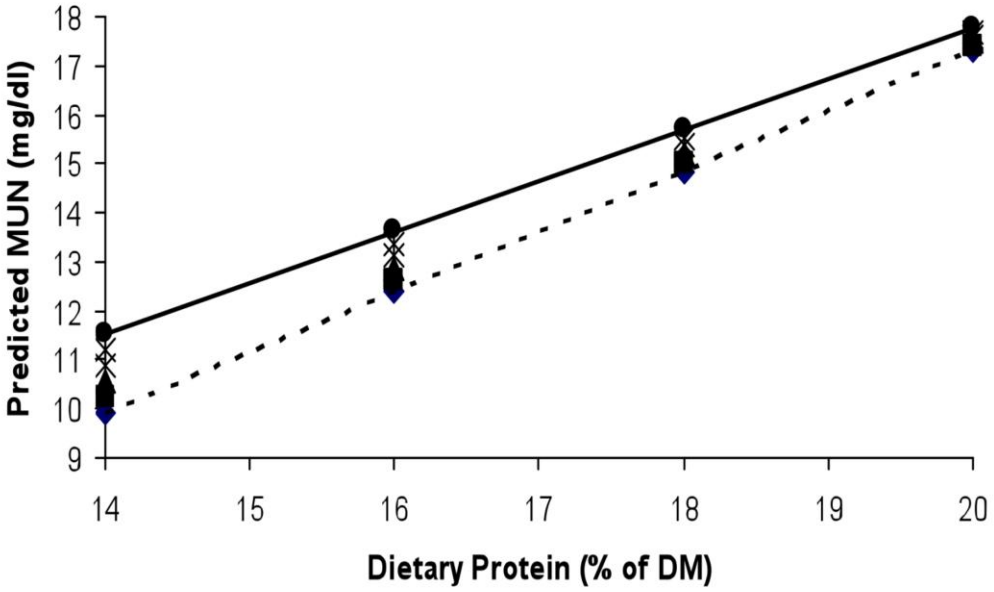

Table 2. 1 A summary of intake, dietary nutrients, and production values for cows from the Cyriac et al. (2008) and Rius et al. (2010) experiments.

| Variable | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|
| Intake | ---------- DM Basis ---------- | | | |
| DMI, kg/d | 24.8 | 3.39 | 13.1 | 34.9 |
| Nitrogen, kg/d | 0.68 | 0.14 | 0.29 | 1.03 |
| CP, kg/d | 4.23 | 0.89 | 1.78 | 6.45 |
| NDF, kg/d | 9.52 | 2.05 | 4.65 | 17.7 |
| ADF, kg/d | 6.11 | 1.41 | 2.99 | 12.3 |
| Starch, kg/d | 5.97 | 1.12 | 3.32 | 8.31 |
| Ash, kg/d | 1.49 | 0.28 | 0.91 | 2.23 |
| Days in milk | 175 | 71.3 | 35 | 413 |
| Milk, kg/d | 36.9 | 8.53 | 12.6 | 60.7 |
| Milk fat, % | 3.48 | 0.61 | 2.15 | 5.72 |
| Milk protein, % | 3.08 | 0.27 | 2.54 | 4.03 |
| Milk lactose, % | 4.89 | 0.21 | 4.06 | 5.26 |
| MUN, mg/dl | 17.9 | 4.06 | 9.68 | 29.15 |
| Dietary Nutrient | ---------- % of DM ---------- | | | |
| DM | 51.4 | 2.15 | 47.9 | 53.9 |
| CP | 16.9 | 2.31 | 13.6 | 19.9 |
| NDF | 38.3 | 5.69 | 31.4 | 50.7 |
| ADF | 24.5 | 4.09 | 18.6 | 35.2 |
| Starch | 24.1 | 3.39 | 14.8 | 28.8 |
| Ash | 6.05 | 0.88 | 4.92 | 7.20 |

Table 2. 2 A summary of production and dietary factors for field trial cows.

| Variable | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|
| Days in milk | 198 | 157 | 2 | 977 |
| Milk, kg/d | 37.8 | 10.0 | 7.4 | 78.6 |
| Milk fat, % | 3.76 | 0.88 | 1.5 | 8.5 |
| Milk protein, % | 3.04 | 0.34 | 2.2 | 5.6 |
| MUN, mg/dl | 15.5 | 3.9 | 4 | 35 |
| Dietary Nutrient | ---------- % of DM ---------- | | | |
| DM | 48.9 | 4.7 | 43.4 | 57.8 |
| CP | 17.6 | 1.6 | 14.4 | 23.4 |
| NDF | 35.3 | 3.4 | 30.5 | 41.5 |
| ADF | 21.2 | 2.4 | 17.6 | 25.5 |
| Ash | 7.1 | 0.6 | 6.0 | 9.7 |
| $NE_L$, mcal/kg | 1.68 | 0.06 | 1.54 | 1.75 |

Table 2. 3 Parameter estimates for a mixed model relating MUN (mg/dl) to nutrient intake, production, and cow factors for the trials summarized in Table 2. 1.

| Effect | Estimate | SE | P< |
|---|---|---|---|
| Intercept | 6.89 | 6.76 | 0.31 |
| ADF Intake (kg/d) | -6.38 | 1.47 | <0.0001 |
| CP Intake (kg/d) | 20.3 | 2.50 | <0.0001 |
| Starch Intake (kg/d) | -4.73 | 1.89 | 0.02 |
| Milk Yield (kg/d) | -0.33 | 0.14 | 0.03 |
| Milk Protein Yield (kg/d) | 5.35 | 3.16 | 0.09 |
| Somatic Cell Count (x1000) | -0.002 | 0.0009 | 0.01 |
| ADF Intake (kg/d) x Milk Lactose Yield (kg/d) | 1.54 | 0.69 | 0.03 |
| ADF Intake (kg/d) x Milk Fat Yield (kg/d) | 14.7 | 4.25 | 0.001 |
| ADF Intake (kg/d) x Days in Milk | 0.04 | 0.02 | 0.02 |
| CP Intake (kg/d) x Starch Intake (kg/d) | -1.33 | 0.36 | 0.0005 |
| CP Intake (kg/d) x Milk Lactose Yield (kg/d) | -2.93 | 1.08 | 0.009 |
| CP Intake (kg/d) x Days in Milk | -0.01 | 0.005 | 0.04 |
| NDF Intake (kg/d) x Starch Intake (kg/d) | 2.02 | 0.71 | 0.006 |
| NDF Intake (kg/d) x Milk Fat Yield (kg/d) | -10.5 | 2.99 | 0.0008 |
| NDF Intake (kg/d) x Days in Milk | -0.03 | 0.01 | 0.03 |
| Starch Intake (kg/d) x ADF Intake (kg/d) | -2.62 | 1.04 | 0.01 |
| Starch Intake (kg/d) x Milk Lactose Yield (kg/d) | 1.25 | 0.72 | 0.09 |
| Starch Intake (kg/d) x Milk Fat Yield (kg/d) | 2.12 | 0.72 | 0.004 |
| Starch Intake (kg/d) x Days in Milk | 0.01 | 0.004 | 0.0007 |
| | | | |
| Random Effects | | | |
| Cow | | | 0.0004 |
| | | | |
| Model Precision | | | Value |
| AIC[1] | | | 750 |
| RMSE[2] | | | 1.77 |

[1] AIC = Akaike Information Criterion
[2] RMSE = Root Mean Square Error

Table 2. 4 Parameter estimates for a mixed model relating MUN (mg/dl) to dietary nutrient concentrations, production, and cow factors for the trials summarized in Table 2. 1.

| Effect | Estimate | SE | P< |
|---|---|---|---|
| Intercept | 16.3 | 1.61 | <0.0001 |
| Milk Protein Yield (kg/d) | -1201 | 324 | 0.0004 |
| Milk Lactose Yield (kg/d) | 682 | 199 | 0.001 |
| Milk Fat Yield (kg/d) | 93.9 | 40.1 | 0.02 |
| Somatic Cell Count (x1000) | -0.003 | 0.0009 | 0.008 |
| Dietary CP (% of DM) x Milk Protein Yield (kg/d) | 15.4 | 4.23 | 0.0006 |
| Dietary CP (% of DM) x Milk Lactose Yield (kg/d) | -9.07 | 2.59 | 0.0009 |
| Dietary CP (% of DM) x Days in Milk | 0.002 | 0.0009 | 0.03 |
| Dietary NDF (% of DM) x Milk Protein Yield (kg/d) | -45.7 | 13.3 | 0.001 |
| Dietary NDF (% of DM) x Milk Lactose Yield (kg/d) | 25.9 | 8.22 | 0.002 |
| Dietary NDF (% of DM) x Milk Fat Yield (kg/d) | 2.72 | 1.58 | 0.09 |
| Dietary NDF (% of DM) x Days in Milk | -0.001 | 0.0004 | 0.01 |
| Dietary Starch (% of DM) x Milk Protein Yield (kg/d) | 28.6 | 8.03 | 0.0007 |
| Dietary Starch (% of DM) x Milk Lactose Yield (kg/d) | -16.2 | 4.94 | 0.002 |
| Dietary Starch (% of DM) x Milk Fat Yield (kg/d) | -2.40 | 1.24 | 0.06 |
| Dietary ADF (% of DM) x Milk Protein Yield (kg/d) | 81.4 | 23.2 | 0.0008 |
| Dietary ADF (% of DM) x Milk Lactose Yield (kg/d) | -46.0 | 14.3 | 0.002 |
| Dietary ADF (% of DM) x Milk Fat Yield (kg/d) | -5.72 | 2.73 | 0.04 |
| | | | |
| Random Effects | | | |
| Cow | | | 0.008 |
| | | | |
| Model Precision | | | Value |
| AIC[1] | | | 758 |
| RMSE[2] | | | 2.16 |

[1] AIC = Akaike Information Criterion
[2] RMSE = Root Mean Square Error

Table 2. 5 Parameter estimates for a mixed model relating MUN concentration (mg/dl) to dietary nutrients, cow, and herd factors for the data summarized in Table 2. 2.

| Effect | Estimate | SE | P< |
|---|---|---|---|
| Intercept | -166 | 26 | 0.002 |
| Dietary CP, % of DM | 5.4 | 1.1 | 0.0001 |
| Dietary NDF, % of DM | 2.84 | 0.45 | 0.0001 |
| Milk Yield, kg/d | 0.66 | 0.12 | 0.0001 |
| Milk Protein, % | 37.7 | 7.3 | 0.0001 |
| CP x NDF | -0.038 | 0.018 | 0.03 |
| CP x Milk Yield | -0.0194 | 0.0057 | 0.001 |
| CP x Milk Protein | -0.73 | 0.24 | 0.003 |
| NDF x Days in Milk | -0.00005 | 0.00002 | 0.009 |
| NDF x Milk Protein | -0.65 | 0.11 | 0.0001 |
| Milk x Milk Protein | -0.073 | 0.023 | 0.002 |
| | | | |
| Random Effects | | | |
| Herd | | | 0.08 |
| Cow(Herd) | | | 0.0001 |
| | | | |
| Model Precision | | | Value |
| AIC[1] | | | 6959 |
| RMSE[2] | | | 2.42 |

[1] AIC = Akaike Information Criterion
[2] RMSE = Root Mean Square Error

# CHAPTER 3: Effect of Animal Variation on Nitrogen Recycling to the Rumen in Dairy Cattle

## ABSTRACT

Milk urea nitrogen (MUN) and blood urea nitrogen (BUN) are correlated with nitrogen balance and nitrogen excretion, however there is also a genetic component to MUN concentrations. Genetic effects on MUN concentrations may be associated with differences among urea transporters in the kidney and the rumen wall. We hypothesized that when fed a common diet, MUN concentrations would be inversely correlated with gastrointestinal and kidney urea clearance rates. Eight lactating cows with similar milk production but varying MUN concentrations, while on a common diet, were infused with [$^{15}N^{15}N$] urea to determine urea synthesis (UER), gastrointestinal urea entry rate (GER), urinary urea excretion (UUE), and urea N excreted in feces (UFE). Urea clearance rates of by the kidneys and GIT were calculated from UER and GER, respectively, and plasma urea N (PUN). Animals weighed 505 ± 61.9 kg and produced 26.3 ± 4.39 kg of milk/d, with MUN concentrations ranging from 7.33 to 20.8 mg/dl (average of 14.9 ± 2.06 mg/dl). Nitrogen intake and fecal N output averaged 512 ± 60.7 g/d and 139 ± 26.4 g/d respectively. Urea entry rate was positively correlated with GER, but not with N intake (due to minimal dietary N variation), indicating GER is a significant determinant of UER. Further, UER was also positively correlated with PUN indicating PUN variation is driven by UER. Plasma urea N tended to be correlated with GER, and PUN and MUN were positively correlated with GIT urea clearance rate. The significant relationships among

GIT urea clearance rate, MUN, and PUN supports the hypothesis that differences in urea transport activity are reflected in MUN concentration. The relationship between GER and PUN also indicates that changes in clearance rate did not totally compensate for changes in urea concentrations resulting in some variation in flux.
Key Words: Urea, gut entry rate, recycling

## INTRODUCTION

Reduced nitrogen (N) excretion on commercial dairy operations has become an important area of interest due to the desire to reduce the environmental impact of the industry. The manure produced by commercial dairy operations emits N into the environment, which can either volatilize or leach into ground and surface water (FAO, 2006, Gay, 2009, USEPA, 1963, 1977). Volatilized ammonia or nitrates leached into water are an environmental hazard and pose a significant human health risk (NRC, 2003). Many studies have shown that dietary manipulation, especially a decrease in CP content, is the most efficient method to reduce the ammonia emissions from dairy operations (Broderick, 2003, Colmenero and Broderick, 2006, Cyriac et al., 2008, Reynal and Broderick, 2005). Reynal and Broderick (2005) observed that reduction of dietary ruminally degradable protein (RDP) content from 13.2 to 10.6% of DM had no effect on milk yield and resulted in a linear decrease in milk protein content, MUN, and BUN. However, reducing RDP too far will compromise rumen fermentation and cause a decrease in DMI and a drop in milk production, which is indicative of a protein deficiency (Cyriac et al., 2008).

Ruminally degradable protein is a major source of N required for bacterial growth and microbial protein synthesis in the rumen (Huntington, 1999, Parker et al., 1995). Rumen microbes, however, can also obtain nitrogen to support growth via transfer of urea from blood to the digestive tract (Huntington, 1999, Lobley, 2001, Parker et al., 1995, Reynolds and Kristensen, 2008). Thus, urea recycling to the rumen is beneficial from a nutrient excretion standpoint as it recovers nitrogen destined for urinary excretion.

Excess dietary protein is catabolized by microbes or the body, and the liberated nitrogen is converted to urea in the liver and released into blood before excretion in urine. Blood urea nitrogen is highly correlated with milk urea nitrogen (MUN; Broderick and Clayton, 1997) and both are correlated with the balance of protein in the animal (Kohn et al., 2002) and nitrogen excretion in urine (Jonker et al., 1998). Thus MUN can be used as a tool to monitor feed management and nitrogen emissions on dairy operations (Jonker et al., 1998).

It has been proposed that cows should have MUN concentrations of 12 mg/dl or less if they are being fed properly which will minimize waste nitrogen and ammonia emissions (Jonker et al., 1999, Simpson, 2009). This target was chosen because it is believed that at this particular MUN concentration milk production will not be affected (Simpson, 2009). However, the field trial data analyzed in chapter 2 demonstrates significant variation in MUN among cows within a herd and among herds after dietary factors had been considered (Chapter 2). Increased DMI, deviations in forage protein, or improperly mixed rations can contribute to excess N production and variation in observed MUN concentration. Additionally, other factors such as BW, stage of lactation, parity, and breed can cause deviations in expected MUN concentration (DePeters and Cant, 1992, Kauffman and St-Pierre, 2001). The effect of cow on MUN variation observed in

chapter 2, may be partially explained by the heritability of MUN among dairy cattle. Heritability values for MUN reported in the literature range between 0.14 and 0.59 (Miglior et al., 2007, Mitchell et al., 2005, Stoop et al., 2007, Wood et al., 2003), and thus genetics may be responsible for the animal and herd variation observed in the field trial.

Blood urea nitrogen concentrations are a function of urea synthesis, urea excretion in urine, and transport into the gut (Figure 3. 1). Because N intake and milk yield were included in the model, it is unlikely that urea synthesis could be the source of variation among cows. However, urea transporters in the kidney and the rumen wall could be the source of the animal variation (Marini et al., 2004, Marini and Van Amburgh, 2003). Differences urea transport into the rumen could impact microbial protein synthesis (Marini et al., 2004, Marini and Van Amburgh, 2003). If urea transport is poor, MUN will be high and the cow may be more susceptible to nitrogen deficiencies in the rumen, which may compromise microbial growth. Conversely, if urea transport is efficient, MUN will be low, and the cows should transport more urea into the rumen, which would reduce susceptibility to low dietary concentrations of ruminally degradable protein. Thus it may not be possible for all cows within a herd or all herds to achieve MUN values of 12 mg/dl or less without causing a ruminal nitrogen deficiency.

Our hypothesis was that cows with low MUN concentrations have increased urea transport activity into the rumen as compared to cows with high MUN concentrations. The objective of this trial was to determine the relationship between MUN and urea entry rate (UER), gastrointestinal tract (GIT) urea entry rate (GER), urinary urea excretion (UUE), urea fecal excretion (UFE), kidney urea clearance rate, and GIT urea clearance rate.

## MATERIALS AND METHODS

### *Animals and Diets*

All animal work was conducted at the Virginia Tech dairy facilities and was approved by the Virginia Tech Animal Care and Use Committee. Eight multiparous cows (4 Jersey and 4 Jersey x Holstein crossbreds) averaging 211 DIM, 2.25 parity, and 26.5 kg of milk/d were selected for the study. Cows were selected to maximize the range in MUN based on average MUN from the prior 2 test days while on a common diet.

Cows were moved from the main free-stall unit to individual tie-stalls in the metabolism barn prior to the start of the study. The experiment lasted 5 d including 2 d for adaptation to the metabolism barn and 3 d for isotope measurements. The experimental diet was the same as that fed to the milking herd (Table 3. 1), and thus no diet adaptation period was required. Cows were fed once daily from d 1 through d 4. On d 5 cows were fed at 4 h intervals to minimize variation in absorbed N. Cows were milked 2x/d and milk weights were recorded at each milking starting on d 3. Animal health and disposition were monitored daily throughout the study, and cows had free access to fresh water.

### *Infusions*

A solution of [$^{15}$N$^{15}$N]urea was prepared by dissolving 2 g of [$^{15}$N$^{15}$N]urea in 2L of saline followed by filter sterilization (0.22 μm filter), and storage at 4$^{\circ}$C. Indwelling jugular catheters (Micro-Renathane polyvinyl, 2.3mm OD x 1.2mm ID, 0.9144 m in length) were inserted into each jugular vein on d 3 of the study. At least 45cm was advanced into the jugular vein. One catheter was used to infuse [$^{15}$N$^{15}$N] urea (99%

enriched) and the contralateral one was used for sample collection. The urea was infused using a clinical infusion pump (Abbott LifeCare 5000) at a rate of 0.4 mmole of urea per hour from 2100h on d 3 through 1900h on d 5. All catheters were pretreated with heparin prior to insertion and filled with heparin (100 IU/ml) when not in use.

***Sample Collection and Analyses***

Samples of TMR and orts were taken on d 4 and 5 and stored at -20°C until analysis. TMR and ort samples were composited and dried to a constant weight at 55°C in a forced-air oven for DM determination. Dried samples were ground in a Wiley Mill (A.H. Thomas, Philadelphia, PA) through a 1-mm screen and submitted to a commercial laboratory (Dairyland Laboratory, Inc.) for nutrient analyses. Kjeldahl N, ether extract, ash, and DM contents were determined according to AOAC methods (1997). Acid detergent fiber and lignin concentrations were determined according to AOAC method 973.18 (1997), and NDF concentration according to Van Soest et al. (1991). Starch was measured as dextrose after treating samples with glucoamylase using a YSI 2700 Select Biochemistry Analyzer (Application Note #319, Yellow Springs, OH). Minerals were quantified according to AOAC method 985.01 (1997) using an inductively coupled plasma spectrometer (Thermo Jarrell Ash, Franklin, MA). Nutrient composition values are presented in Table 3. 1 as averages of experimental diet sample analyses and calculated nutrient composition values based on the individual ingredient analyses and dietary inclusion rates.

Milk samples were collected starting on d2 and analyzed by the United Federation of DHIA Laboratory (Blacksburg, VA) for true protein, fat, and lactose concentrations and SCC using a Fossomatic 4000 Combi infrared analyzer (Foss, Eden Prairie, MN). Milk urea nitrogen concentrations were determined using a modified Berthelot procedure

(ChemSpec 150 Analyzer; Bentley Instruments, Chaska, MN). Total daily milk and milk component yields were calculated by summation of AM and PM values.

Urinary catheters were inserted on d 3 of the study. An 8-gauge Foley catheter was advanced into each cow and connected to a 5 gallon plastic container containing $H_2SO_4$ equivalent to 1% of total daily excreted urine to keep the pH under 2.5. Urine was composited every 12 h by cow on d 3 and 4 and samples were frozen at -20°C until analysis. On d 5 composite urine samples were collected during the last 12 h of infusion and frozen at -20°C until $^{15}N^{15}N$ enrichment analysis. Urine samples collected during the last 12 h of infusion were analyzed for urea-N (UUN; Stanbio Urea Nitrogen Kit 580, Stanbio Laboratory, Inc.; Table 3. 2) and $^{15}N^{15}N$ enrichment as previously described (Sarraseca et al., 1998).

Feces were composited every 24 h by cow and subsampled (500 g) from d 3 through d 5. Fecal samples were freeze dried (FreeZone Plus 6; Labconco, Kansas City, MO) to a constant weight for DM determination. Dried samples were then ground in a Wiley mill through a 1-mm screen and submitted to a commercial laboratory (Dairyland Laboratory, Inc.) for analysis of CP, soluble protein, NDF and ADF, starch, fat, and ash (Table 3. 3).

On d 5, blood samples were collected from each cow every 2 h over a 12 h period. into 10ml, heparin coated vacuutainer tubes and centrifuged at 3,000 rpm for 5 minutes at 4°C to collect plasma. Plasma was stored at -20°C until analysis. Plasma samples were pooled by animal and analyzed for urea-N (PUN; Stanbio Urea Nitrogen Kit 580, Stanbio Laboratory, Inc.).

***Calculations and Statistical Analyses***

Urea entry rate into blood (Figure 3. 1), UUE, GER, UFE, return of urea N from the rumen to the ornithine cycle (ROC), and urea utilized for anabolism by the body (UUA; all g urea N/h; Table 3. 2) were calculated from isotopic enrichment and urinary output as previously described (Lobley et al., 2000). The fractions of synthesized urea eliminated in the urine (u) or transferred to the GIT (1-u), and the resultant sub fractions of 1-u that were returned to the ornithine cycle (r), excreted in feces (f), or utilized for anabolism (a; all g/g; Table 3. 2) were also calculated from isotopic enrichment and urinary output as previously described (Lobley et al., 2000). The clearance rate of urea across the kidney (kidney urea clearance) and GIT (GIT urea clearance) were calculated as:

Kidney urea clearance (L/h) = UUE / (PUN/1000*10),

and

GIT urea clearance (L/h) = GER / (PUN/1000*10),

where PUN had units of mg urea N/dl (Table 3. 2).

Data were analyzed using the MIXED procedure of SAS (Version 9.2, SAS Institute Inc., Cary, NC). Simple regression analyses were performed to examine relationships between MUN, PUN, UER, GER, urea clearance, and the various observed values (Table 3. 4). Unless otherwise states, significance was declared at $P < 0.05$.

## RESULTS AND DISCUSSION

Cows used in the study averaged 26.3 kg of milk per day with dry matter intakes of 18.2 kg/d (Table 3. 2). Dietary CP content and intake averaged 17.6% (DM basis) and 3.19 ± 0.38 kg/d, respectively. Fecal N excretion (Table 3. 3) was consistent with prior

observations that used a similar diet (Martinez et al., 2009). Milk urea nitrogen averaged 14.9 mg/dl, which is consistent with predictions from the Jonker et al. (1999) model. However MUN means by cow ranged from 11.6 to 17.3 mg/dl underscoring the variation among cows on a common diet. Milk production was similar among animals and no significant correlations were observed among experimental diet, DMI, N intake, BW, milk N, fecal N, MUN, or PUN. Given that there were no relationships among cows in N intake, milk production, BW, or urea excretion, despite variation in urea substrate availability, supports the presence of potential variation in urea transport activity among animals.

Urea synthesis (UER) was positively correlated with PUN concentrations ($P$ = 0.01) and tended to be correlated with MUN concentrations indicating that animals with high PUN concentration had increased urea synthesis, while animals with low PUN concentration had decreased urea synthesis (Table 3. 4). However, because the cows were fed a diet sufficient in RDP according to the NRC (2001), the positive relationship between UER and PUN is probably not causative. If RDP requirements are met, any change in gut entry rate would result in a comparable change in ammonia recycling to the liver, and thus a comparable change in urea synthesis as indicated by the significant positive correlation between GER and UER.

Milk urea N was correlated to PUN (Table 3. 4) as has been previously observed (Broderick and Clayton, 1997). Plasma urea concentration reflects the balance of inputs from urea synthesis, as well as losses to the gut and urine (Figure 3. 1), thus a change in any of those 3 entities would be reflected in PUN. There were no significant correlations between PUN and GER, UUE, or UFE indicating that variation in PUN was not directly associated with urea N losses (Table 3. 4). Given that PUN is a substrate for urea

transport into the rumen (Mugerwa and Conrad, 1971, Norton et al., 1978), it was surprising that that the relationship between PUN and GER was only a positive trend ($P$ = 0.08). The weak correlation between PUN and GER indicates that urea transporter activity may not be constant, which is supported by the strong correlation between GIT urea clearance rate and PUN ($P$ = 0.02; Table 3. 4). If the clearance rates were constant across PUN concentrations, a stronger correlation between PUN and GER would have been observed. Thus, the significant relationship between PUN and GIT urea clearance rate indicates that GER differences are most likely not causing the observed variation in PUN concentrations observed.

Consistent with our hypothesis, GIT clearance rates were negatively correlated with MUN ($P$ = 0.008; Figure 3. 2) and PUN ( $P$ =0.02), indicating that at least a portion of the variation in PUN and MUN was associated with altered clearance rates by the GIT where cows with high MUN had lower clearance rates than cows with low MUN. The negative relationship between MUN and GIT urea clearance rate (Table 3. 4; Figure 3. 2) indicates possible cow variation in urea transporter activity of the gastrointestinal tract which can be caused by differences in the amount of transporter (Vmax), transport affinity (Km), or due to transporter saturation (Cleland, 1967). Because transport rates were changing, it would seem that the transporter was not saturated. Our data are not adequate to differentiate between transporter number and affinity. If variation in urea transport activity is a genetically inherited trait it would be expected that animals, regardless of their age or stage of production, would have the same urea transport activity over a lifetime. A significant effect was observed between ROC on MUN and PUN variation (Table 3. 4), which also supports differences in urea transport activity as ROC causes change in UER and is a downstream effect of PUN and GER.

Kidney urea clearance rate and urea utilization for anabolism were not significantly correlated with MUN or PUN variation. The lack of a significant correlation between kidney urea clearance rates with either MUN or PUN indicates that kidney urea extraction was not the cause of variation observed in PUN and MUN. Urinary urea excretion was not significantly correlated with either MUN or PUN, supporting the observation that kidney urea extraction is not the cause of variation in PUN concentration. The poor relationships among kidney urea clearance rate, UUE, and PUN suggests that UUE may be strictly dictated by N balance and the appropriate rate is achieved regardless of PUN concentration. Since kidney and GIT tissues share a common transporter (Stewart et al., 2004), the two different relationships observed with PUN suggests that variation among urea transporters may not be the driving source causing differences in PUN concentration. Alternatively, there may be unique characteristics of the rumen or additional transporters associated with the rumen causing the observed differences in PUN concentration across cows.

The observed variation of GIT urea clearance rate demonstrated in the present study may have a considerable impact on RDP supply, since urea entering the gut is rapidly converted to ammonia (Chalmers et al., 1971, Tamminga, 1983). Ammonia not utilized by ruminal microbes diffuses across the GIT into the bloodstream where it is carried to the liver and converted into urea (Huntington, 1999, Parker et al., 1995, Reynolds and Kristensen, 2008). Microbial requirements for ammonia in the rumen are relatively low and can be met with a diet containing 13% CP (Satter and Roffler, 1975). Varying transport activity could result in cows that are more susceptible to RDP deficiencies, but such a hypothesis must be tested with diets that vary in RDP content.

# CONCLUSIONS

Variation in MUN among cows on a common diet with similar milk production and no differences in DMI, N intake, BW, milk N, and fecal N may be related to variation in GIT urea clearance rates. This may be useful from a management standpoint, as cows with lower MUN may be less susceptible to an RDP deficiency and could be fed lower protein diets while those with high MUN possibly require higher CP diets. Producers could use this information to better manage their herds using grouping strategies and multiple rations with differing RDP contents. This would result in reduced nitrogen emissions and possibly reduced production costs.

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Figure 3. 1 Flow diagram of urea-N fates within the ruminant. Excess dietary N is synthesized into urea-N within the liver and enters into the blood stream (urea-N entry rate; UER). Urea-N in blood (PUN) exchanges with urea-N in milk (MUN). PUN can either enter the GIT (GIT entry rate; GER) or it can be eliminated in urine (urinary urea excretion; UUE). Urea-N that enters the GIT can be lost in feces, converted into microbial protein, or it can be broken down into ammonia (NH3) and synthesized into urea once again.

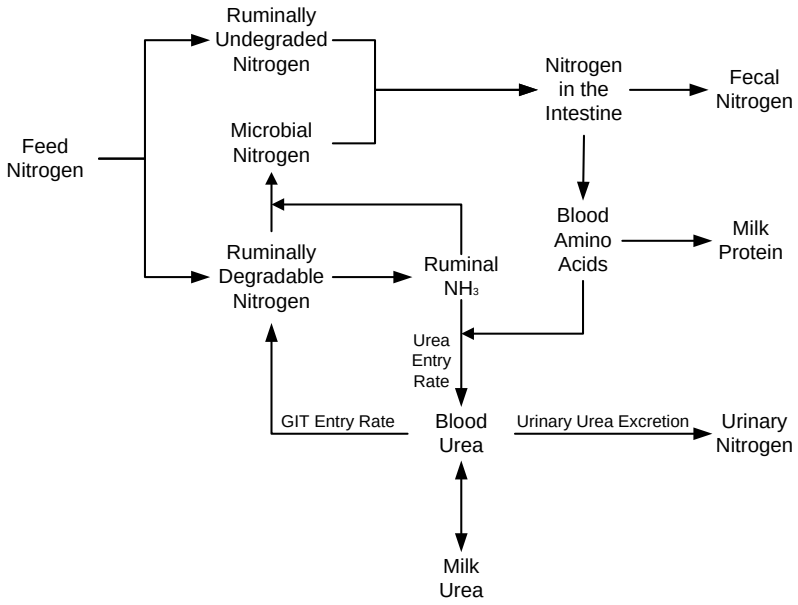

Figure 3. 2 Gastrointestinal and kidney urea clearance rates versus the mean observed MUN during the 3 d infusion of [$^{15}N^{15}N$]urea. Slope, $R^2$, and *P*-value associated with GIT urea clearance rate was y = -7.31x + 191, $R^2$ = 0.72, and *P* = 0.008. Slope, $R^2$, and *P*-value associated with kidney urea clearance rate was y = -1.19x + 59.9, $R^2$ = 0.05, and *P* = 0.61.

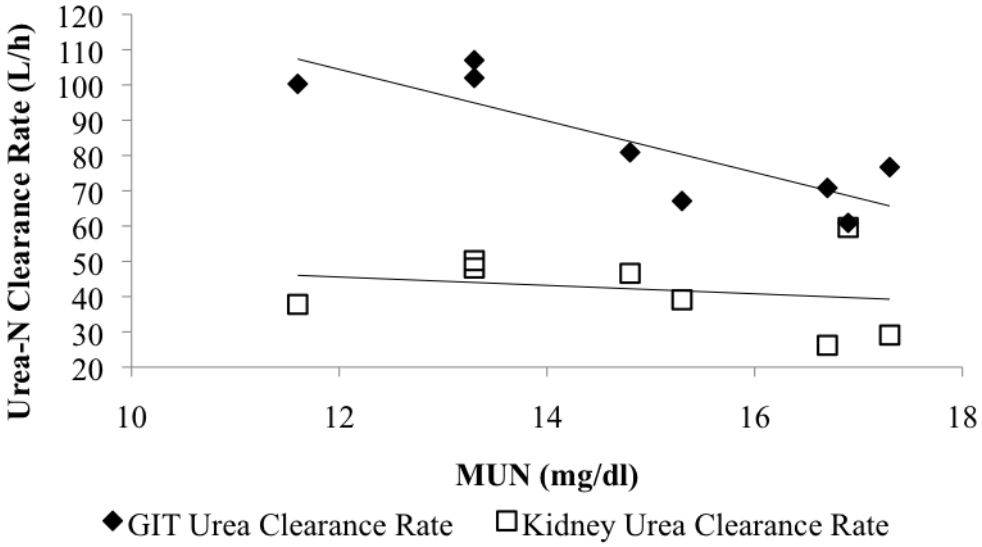

Figure 3. 3 Gastrointestinal entry rate (GER) and return to ornithine cycle (ROC) versus the mean observed PUN during the 3 d infusion of [$^{15}N^{15}N$]urea. Slope, $R^2$, and *P*-value associated with GER was y = 0.33x + 8.43, $R^2$ = 0.42, and *P* = 0.02. Slope, $R^2$, and *P*-value associated with ROC was y = 0.45x + 10.5, $R^2$ = 0.67, and *P* = 0.01.

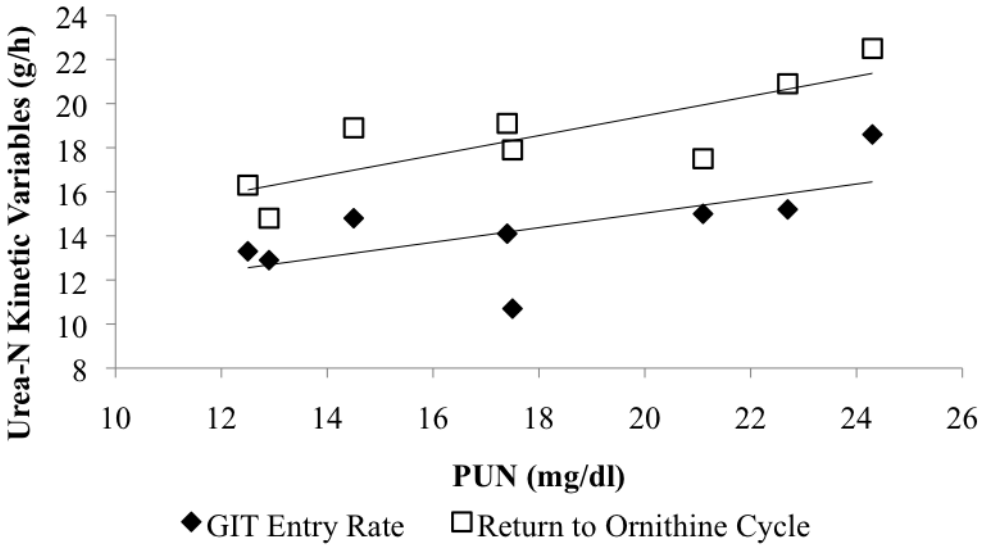

Table 3. 1 Ingredient composition of TMR and components of TMR and grain mix.

| Nutrient | % of DM |
|---|---|
| TMR components | |
| Corn silage | 44.4 |
| Grain mix | 27.4 |
| Corn meal | 12.3 |
| Mixed grass/alfalfa silage | 10.7 |
| Alfalfa hay, pre-bloom | 5.25 |
| Grain mix components | |
| SBM, 48% | 34.2 |
| Citrus Pulp Dehydrated | 26.4 |
| Distillers Ethanol | 6.81 |
| Pro-Lak | 6.59 |
| Wheat Middlings | 6.45 |
| Limestone | 3.94 |
| Molasses Dehydrated | 3.40 |
| Animal Fat | 2.87 |
| Sodium bicarbonate | 2.15 |
| Megalac plus | 2.11 |
| Urea, 45% | 1.69 |
| Salt, white | 1.08 |
| Dyna-Mate | 1.06 |
| Magnesium oxide | 0.71 |
| Availa-4 | 0.20 |
| Vitamin ADE mix[1] | 0.17 |
| Selenium, 0.06% | 0.13 |
| Vitamin E 60000 | 0.70 |
| Rumensin 90 | 0.02 |
| Nutrient concentrations of TMR | % of DM |
| DM | 42.5 |
| CP | 17.6 |
| Protein Solubility, % of CP | 37.2 |
| Starch | 23.7 |
| NFC | 40.3 |
| NDF | 29.7 |
| ADF | 20.9 |
| Lignin | 3.79 |
| Fat | 5.8 |
| Ash | 7.48 |

[1]Contained (% DM) Vitamin A, 26,485 KIU/kg; Vitamin D, 8,828 KIU/kg; Vitamin E, 44,141 mg/kg.

Table 3. 2 Mean production and N metabolism values for animals with high (>13.5 mg/dl) or low (<13.5 mg/dl) milk urea nitrogen concentrations (MUN) during trial.

| Variable | High MUN | Low MUN | SE | P-Value |
|---|---|---|---|---|
| Production | | | | |
| BW, kg | 499 | 513 | 46.9 | 0.78 |
| DMI, kg/d | 18.0 | 18.3 | 1.64 | 0.87 |
| N intake, g/d | 508 | 516 | 46.3 | 0.87 |
| Milk, kg/d | 28.9 | 23.7 | 2.57 | 0.08 |
| Milk lactose, kg/d | 1.38 | 1.07 | 0.14 | 0.08 |
| Milk protein, kg/d | 0.95 | 0.81 | 0.06 | 0.07 |
| Milk fat, kg/d | 1.68 | 1.30 | 0.16 | 0.06 |
| Milk nitrogen, g/d | 150 | 128 | 10.0 | 0.07 |
| Urine Excretion | | | | |
| Urine, L/d | 21.4 | 23.0 | 2.65 | 0.57 |
| Urine N, g/d | 49.1 | 52.8 | 6.09 | 0.56 |
| Urea-N concentrations | | | | |
| MUN, mg/dl | 15.6 | 14.2 | 1.45 | 0.35 |
| PUN, mg/dl | 18.9 | 16.8 | 3.31 | 0.53 |
| Urinary urea, g/d | 32.4 | 33.4 | 6.63 | 0.88 |
| Urea-N kinetics (g/h) | | | | |
| Urea entry rate (UER) | 21.3 | 21.9 | 1.91 | 0.76 |
| GIT entry rate (GIT) | 14.3 | 14.4 | 1.74 | 0.98 |
| Urinary urea excretion (UUE) | 6.98 | 7.57 | 1.38 | 0.69 |
| Urea-N to fecal excretion (UFE) | 0.05 | 0.05 | 0.005 | 0.96 |
| Return to ornithine cycle (ROC) | 18.2 | 18.8 | 1.84 | 0.76 |
| Urea-N to anabolism (UUA) | 4.45 | 4.89 | 0.52 | 0.43 |
| Fractional transfers (g/g) | | | | |
| UER to urine (u) | 0.33 | 0.34 | 0.06 | 0.79 |
| UER to GIT (1-u) | 0.67 | 0.66 | 0.06 | 0.79 |
| GER to ROC (r) | 1.30 | 1.31 | 0.13 | 0.96 |
| GER to feces (f) | 0.004 | 0.003 | 0.001 | 0.73 |
| GER to UUA (a) | 0.32 | 0.34 | 0.06 | 0.79 |
| Urea clearance rates (L/h) | | | | |
| GIT | 77.2 | 89.1 | 12.4 | 0.37 |
| Kidney | 38.2 | 45.9 | 7.94 | 0.36 |

Table 3. 3 Observed fecal output.

| Nutrient | g/d | SD |
|---|---|---|
| DM | 4740 | 1000 |
| Nitrogen | 139 | 26.4 |
| ADF | 1570 | 455 |
| NDF | 2270 | 556 |
| Lignin | 467 | 126 |
| Starch | 68.0 | 21.3 |
| Fat | 126 | 41.6 |
| Ash | 633 | 136 |

Table 3. 4 Variables that made significant contributions to the regression of MUN, PUN, UER, and GER on multiple factors using the mixed effects model.

| Dependent Variable | Independent Variable | Slope | SE | P-Value |
|---|---|---|---|---|
| MUN | PUN | 0.38 | 0.11 | 0.01 |
| | Urea entry rate (UER) | 0.53 | 0.25 | 0.08 |
| | Return to ornithine cycle (ROC) | 0.54 | 0.26 | 0.08 |
| | GIT urea clearance rate | -0.09 | 0.03 | 0.008 |
| PUN | BW | -0.05 | 0.02 | 0.09 |
| | GIT entry rate (GER) | 1.28 | 0.61 | 0.08 |
| | Urea entry rate (UER) | 1.45 | 0.42 | 0.01 |
| | Return to ornithine cycle (ROC) | 1.50 | 0.44 | 0.01 |
| | GIT urea clearance rate | -0.19 | 0.07 | 0.02 |
| UER | GIT entry rate (GER) | 0.79 | 0.32 | 0.05 |
| | Return to ornithine cycle (ROC) | 1.04 | 0.004 | <0.0001 |
| | Urea-N to anabolism (UUA) | 2.89 | 0.82 | 0.01 |
| GER | Return to ornithine cycle (ROC) | 0.67 | 0.27 | 0.05 |
| | GER to feces (f) | -1790 | 708 | 0.05 |
| | Kidney urea clearance rate | -0.15 | 0.06 | 0.04 |

**CHAPTER 4: Effect of Simultaneous Reduction of Ruminally Degradable Protein and Ruminally Undegradable Protein Below NRC Requirements on Milk Production in Dairy Cattle**

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## ABSTRACT

Previous studies have shown that ruminally degradable protein (RDP) and ruminally undegradable protein (RUP) can be reduced independently below NRC requirements, with no effect on milk production or animal health, suggesting requirements may exceed true needs. However, because some of the RDP requirement is met by urea recycling which is dependent on overall protein supply, reducing both RDP and RUP simultaneously could induce an RDP deficiency. We hypothesized that dairy cattle may be able to maintain performance when fed a combination of sub-NRC requirement levels of RUP and RDP. Thirty-six mid-lactation dairy cows (24 Holstein and 12 Jersey x Holstein crossbreds) were fed diets containing sufficient or deficient amounts of RDP and RUP in a 2x2 factorial arrangement within a replicated 4x4 Latin Square design with 3-wk periods. Diets were formulated to contain 16.5, 15.75, or 15.0 % CP (DM basis) with RUP and RDP balances of +57 and +58 g/d (High-RUP/High-RDP, 16.5% CP); +42 and -209 g/d (High-RUP/Low-RDP, 15.75% CP); -133 and +61 g/d (Low-RUP/High-RDP, 15.75% CP); or -182 and -186 g/d (Low-RUP/Low-RDP,

15.0% CP), respectively. All diets contained 46.8% forage and 53.2% concentrate on a DM basis. Milk yield and composition were measured and microbial purine output was calculated from urinary concentrations of allantoin and uric acid. Fecal output and total tract apparent digestibilities of nutrients were estimated using DMI, diet indigestible NDF (INDF), and fecal INDF. Treatment had no effect on milk production, milk composition, N balance, or BW differences among animals. Due to dietary deviations, treatments containing low RUP content significantly reduced animal intakes of DM, N, INDF, lignin, starch, NFC, and ash; and excretion of MUN, urinary urea N, and fecal N. Total tract apparent digestibility of nutrients and N efficiency were significantly increased in diets containing low levels of RUP, which was likely caused by reduced DMI. Microbial N flow, calculated from urinary purine output, was not significantly affected by treatment. Reduced levels of dietary RUP and RDP reduced N excretion and improved N efficiency without altering microbial outflow.
Key words: ruminally degradable protein, ruminally undegradable protein, nitrogen requirement, milk production

## INTRODUCTION

Ruminal bacteria require carbohydrates and nitrogenous substrates such as protein, peptides, amino acids, and ammonia for growth and production of microbial protein (NRC, 2001, Russell et al., 1992). Ruminally synthesized microbial protein, ruminally undegradable protein (RUP), and endogenous protein contribute to available metabolizable protein (MP), which dairy cattle utilize to meet amino acid requirements (Satter and Roffler, 1975). Amino acids are required to synthesize proteins essential for

maintenance, growth, reproduction, and lactation. The goals of ruminant protein nutrition are to provide sufficient dietary protein for maximal production and minimal loss of N to the environment, and to minimize protein feeding to reduce production costs.

Dairy producers in the U.S. tend to feed protein above NRC requirements (Hristov et al., 2006, Jonker et al., 2002). On average lactating dairy cattle transfer 25 to 30% of consumed N to milk or tissue N, while the remainder is excreted in urine and feces (Bequette et al., 2003, Wilkerson et al., 1997). Feeding protein above NRC requirements therefore results in increased urinary and fecal N excretion. Accumulation of manure N is an environmental hazard as N can be converted to ammonia or leached into ground water during manure collection and storage (Varel et al., 1999). Volatilized ammonia results in reduced air quality and N leaching results in eutrophication, which pose a significant risk to human health (James et al., 1999, NRC, 2003, USEPA, 2004). Various studies have observed that dietary manipulation, in particular a decrease in CP, is the most efficient method to reduce ammonia emissions from dairy operations (Broderick, 2003, Colmenero and Broderick, 2006b, Reynal and Broderick, 2005).

Studies performed by Cyriac et al. (2008) and Agle et al. (2010) observed that lactational performance could be maintained with RDP levels below those recommended in the current NRC (2001). However, previous trials that fed decreasing concentrations of RDP have observed linear declines in milk production, as RDP contributes to microbial protein synthesis and therefore available MP supply (Herrera-Saldana and Huber, 1989, Kalscheur et al., 2006). Additionally, Rius et al. (2010) and Davidson et al. (2003) observed no effect on milk production when lactating dairy cattle were fed RUP levels below NRC recommendation (2001). Current NRC recommendations for RDP and RUP are based on a regression approach using previously available literature, where few

of the studies evaluated varying levels of RDP and RUP below NRC requirements. It is possible that current requirements for RDP and RUP are set too high due to insufficient data used to derive the current NRC protein model. However, since NRC prediction equations are based on cow averages from previous literature, it is unlikely that insufficient data profoundly influences the precision of the NRC model.

Another possible reason for over-estimation of RDP and RUP requirements in the current dairy NRC may be the inclusion of urea recycling as a constant function among animals. Chapter 3 of the present thesis used lactating cows with intrinsically high or low MUN concentrations, and observed variation in urea transport activity indicating that urea recycling differs amongst cows. Additionally, urea recycling is known to vary with level of protein feeding and ruminally available carbohydrate (Broderick and Clayton, 1997). Thus, diets containing high concentrations of ruminally available carbohydrate may increase protein requirement above NRC recommendations if diets are low in RDP and urea transport into the rumen is poor, which is currently a relationship that is not reflected in the NRC model. The advancement of scientific research has resulted in additional information, such as variation in urea recycling and level of carbohydrate in the diet, which may play an important role in the calculation of RDP and RUP requirements for lactating dairy cattle, and therefore must be considered in future dairy NRC protein models.

The objectives of this work were to determine whether both RDP and RUP could be reduced below NRC requirements without compromising milk production, nutrient digestion, and nutrient absorption. It was hypothesized that dairy cattle may be able to maintain performance when fed a combination of sub-NRC requirement levels of RUP and RDP.

## MATERIALS AND METHODS

### *Animals and Diets*

All animal work was conducted at the Virginia Tech dairy facilities and was approved by the Virginia Tech Animal Use and Care Committee. Thirty-six mid-lactation dairy cows (24 multiparous Holstein and 12 multiparous Jersey x Holstein crossbreds) were selected from the Virginia Tech dairy herd. Two ruminally cannulated dry cows (multiparous Jersey x Holstein crossbreds) were used to assess dietary and fecal indigestible neutral detergent fiber (INDF) concentrations. Additionally, two ruminally cannulated mid-lactation (multiparous Jersey x Holstein crossbreds) were used to determine ruminal degradability characteristics of CP for each of the major feed ingredients used in the diets.

Cows used in the feeding trial were housed in a freestall barn at the Virginia Tech dairy complex and individually fed using a Calan door system (American Calan Inc., Northwood, NH). Animals were milked twice daily at 0230 and 1300h. Animals were balanced in 4 groups based on milk production, DIM, breed, and dry date, and randomly assigned to 1 of 4 treatment sequences. Seven cows were removed from the study due to death (n=1), displaced abomasum (n=1), milk fever (n=1), and mastitis infections (n=4).

The study was a 4x4 Latin Square design with 4, 21-d periods. Dietary treatments contained either sufficient or deficient amounts of RDP and RUP in a 2x2 factorial arrangement. Diets were formulated to contain 16.5, 15.75, or 15.0 % CP (DM basis) with RUP and RDP balances of +57 and +58 g/d (High-RUP/High-RDP, 16.5% CP); +42 and -209 g/d (High-RUP/Low-RDP, 15.75% CP); -133 and +61 g/d (Low-RUP/High-

RDP, 15.75% CP); or -182 and -186 g/d (Low-RUP/Low-RDP, 15.0% CP), respectively using the NRC (2001) model (Table 4. 2). Dairy cattle meet their MP requirements by various N sources, including RDP, endogenous protein, and RUP. Since a portion of required MP is made up of RUP, dairy cattle therefore have a need for RUP. As a result, RUP is referred to as a requirement for dairy cattle in Table 4. 2, even though it is technically an MP requirement.

Ruminally undegradable and degradable protein were manipulated by varying the amounts of corn grain, soybean meal (48% CP), ruminally protected soybean meal (non-enzymatic browned SBM; West Central Cooperative, Ralston, IA), soybean hulls, urea, and tallow while holding forages, minerals, and vitamins constant. Diets were formulated to meet all other nutrient requirements according to NRC (2001) recommendations for a mid-lactation dairy cow weighing 612 kg, 70 DIM, and producing 36.3 kg of milk/d containing 3.5% fat and 3.0% protein and consuming 22.4 kg/d of DM. Final diets contained 46.8% forage and 53.2% concentrate on a DM basis. Feeding rates were adjusted weekly to maintain constant DM proportions. Diets were mixed at 1200 h and fed once daily as a TMR. Feed offered each day was adjusted to achieve a target of 10% refusal.

***Sample Collection and Analyses***

Feed intake and refusals were recorded daily. Samples of feed, milk, urine, and feces were obtained during the last week of each treatment period (the collection week). During collection weeks TMR samples were collected twice, while samples of forages and concentrates were obtained once. All samples were frozen at -20°C until analysis. Feed samples were composited by diet and period and then subsampled (Behnke K, 1996). Subsamples were dried to a constant weight at 55°C in a forced-air oven for DM

determination. Dried samples were ground in a Wiley Mill (A.H. Thomas, Philadelphia, PA) through a 2-mm screen and submitted to Dairyland Laboratories, Inc. (Arcadia, WI) for nutrient analyses. Kjeldahl N, ether extract, ash, and DM contents were determined according to AOAC methods (AOAC, 1997). Acid detergent fiber and lignin concentrations were determined according to AOAC method 973.18 (1997), and NDF concentration according to Van Soest et al. (1991). Starch was measured as dextrose after treating samples with glucoamylase using a YSI 2700 Select Biochemistry Analyzer (Application Note #319, Yellow Springs, OH). Minerals were quantified according to AOAC method 985.01 (AOAC, 1997) using an inductively coupled plasma spectrometer (Thermo Jarrell Ash, Franklin, MA). Indigestible NDF (INDF) was used as an intrinsic digestibility marker and analyzed as described by Huhtanen et al. (1994), except that 50-μm pore sized bags (Ankom Technology) were used for the ruminal incubation. Composition and ingredient inclusion rates of diets are presented in Table 4. 1. The average values of observed and predicted nutrients are presented in Table 4. 2.

Milk weights, milk composition, and BW were recorded automatically at each milking (AfiFarm Herd Management Software, S.A.E. AfiKim, Kibbutz Afikim, Israel). Milk samples were taken at each milking on three separate days during the last 7 d of each period. Milk samples were submitted to United Federation of DHIA (Blacksburg, VA) for determination of milk true protein, fat, lactose, SNF, and SCC (AOAC, 1997) using a Fossomatic 4000 Combi infrared analyzer (Eden Prairie, MN). Concentrations of MUN were determined using a modification of the Berthelot procedure (ChemSpec 150 Analyzer; Bentley Instruments, Chaska, MN). Daily milk composition was calculated from the weighted a.m. and p.m. observations. Milk N content was calculated by dividing milk protein by 6.38.

Spot urine samples (200 ml per sampling) were collected at 1330 h on the 2nd day of the last week of each period by massaging the vulva. Urine samples were placed on ice and transported to the laboratory. Subsamples of urine were acidified with 0.072N $H_2SO_4$ at a ratio of 1:1 to achieve a pH <2.5, and then frozen at -20°C until analysis. Urine samples were analyzed for allantoin (Chen, 1992), uric acid (Stanbio Uric Acid Kit 1045, Stanbio Laboratory, Inc., San Antonio, TX), urea-N (UUN; Stanbio Urea Nitrogen Kit 0580, Stanbio Laboratory, Inc.), and creatinine (Stanbio Creatinine Kit 0420, Stanbio Laboratory, Inc.). Total urine N concentration was determined on a vario EL cube CN analyzer (Elementar Americas Inc., Mount Laurel, NJ). Daily urine volume and excretion of UUN and total N were estimated from urinary creatinine concentration and BW, assuming a creatinine excretion rate of 29mg/kg of BW (Valadares et al., 1999). Urinary purine derivatives (PD; mmol/d) were calculated from the summation of weighted urine allantoin and uric acid concentrations. Total absorption of microbial purines and ruminal synthesis of microbial N (g/d) was calculated as described by Chen et al. (1992).

Spot fecal samples (400 g per sampling) were collected from the rectum at the time of urine collections, and frozen at -20°C until analysis. Fecal samples were composited per animal and sampling period, and freeze dried (FreeZone Plus 6; Labconco, Kansas City, MO) to a constant weight to determine DM content and for further analyses. Dried samples were ground in a Wiley Mill (A.H. Thomas, Philadelphia, PA) through a 2-mm screen and analyzed for NDF, ADF, and INDF. Indigestible NDF and total N concentrations in feces were analyzed as previously described for TMR and urine samples, respectively. Fecal CP% was calculated by

multiplication of total N by 6.25. Fecal output and total tract apparent digestibility of nutrients were calculated from DMI, diet INDF, and fecal INDF:

Fecal DM output (kg/d) = (DMI (kg/d) × Diet INDF %) ÷ Fecal INDF %,

Fecal Nutrient output (kg/d) = Fecal DM output x Fecal Nutrient % ÷ 100,

Apparently digested nutrient (kg/d) = Nutrient Intake (kg/d) – Fecal Nutrient Output (kg/d),

and

Digestibility (%) = (Apparently digested Nutrient ÷Nutrient Intake) × 100.

Nitrogen balance and N efficiency were calculated as:

N Balance (g/d) = intake N (g/d) – (milk N (g/d) + urine N (g/d) + fecal N (g/d))

and

N efficiency (%) = (milk N (g/d) / intake N (g/d)) × 100.

***In Situ Study***

To assess dietary and fecal indigestible neutral detergent fiber (INDF) concentrations, two ruminally cannulated, dry, nonpregnant cows (multiparous Jersey-Holstein crossbreds) were used. The cows were housed in individual pens equipped with feeders and automatic waterers. Cows were fed a lactating cow TMR containing 17.9% CP (DM basis) twice daily ad libitum. Samples of TMR from each treatment and individual fecal samples were dried at 55°C and ground to 2mm. Approximately 5 g of sample was placed in 5 × 10 cm polyester bags (Ankom Technology, Macedon, NY) with a pore size of 50 μm and suspended in the rumen in a large (36 × 42 cm) nylon mesh bag for 288 h. All analyses were conducted in duplicate. After ruminal incubation, the bags were rinsed in cold water, washed using a household washing machine on the knit, cold wash cycle, and immediately dried at 55°C for 48 h. Residues plus a sample of the

original material were ground to 1 mm and analyzed for NDF content as previously described. Indigestible NDF was calculated using the following equations:

Digested NDF (%) = [Original DM (g) x NDF (%) / 100 – residual DM (g) * NFD (%) / 100] / Original DM (g) x NDF (%) / 100,

and

INDF (%) = 100 – Digested NDF (%),

where INDF represents indigestible NDF (%).

Additionally, ruminal degradability characteristics of CP for each major feed ingredient used in the study were determined using two ruminally cannulated, mid-lactation cows (multiparous Jersey-Holstein crossbreds). Cows used to determine ruminal degradation of CP were housed in the freestall barn at the Virginia Tech dairy complex and fed a lactating cow TMR containing 16.7% CP (DM basis) once daily ad libitum. Samples of corn silage, mixed alfalfa and grass silage, alfalfa hay, ground dry corn grain, soybean meal, ruminally protected soybean meal, and soybean hulls were dried at 55°C and ground to 2mm. Approximately 10 g of sample were sealed in 10 × 20 cm polyester bags (Ankom Technology, Macedon, NY) with a pore size of 50 μm and suspended in the rumen in a large (36 × 42 cm) nylon mesh bag.

Samples were placed in the rumen in reverse order and removed simultaneously at the end of the experiment. Duplicate bags resided in the rumen of each cow for 4, 8, 12, 24, and 36 h. A 0-h sample was immersed in 39°C water for 20 min. After incubation all samples were rinsed in cold water, washed in a household washing machine using the knit, cold wash cycle, and dried at 55°C for 48 h. Residues were ground to 1 mm and analyzed for CP content. Crude protein disappearance was calculated as the difference between the original CP mass and the mass remaining after ruminal fermentation and

expressed as a percentage of the orginal CP mass. Digestion rates were calculated using the Proc NLin procedure of SAS as described by NRC (2001):

$$\text{Undegraded protein (\%)} = C + B \times e^{(-k \times t)},$$

where B represents the amount insoluble (%), k represents the degradation rate of B (%/h), t represents time in the rumen (h), and C represents the fraction of undegradable protein as described by the NRC (2001).

***Statistical Analysis***

Means of DMI, nutrient intake, milk yield, milk yield composition, and excretion of urinary N, urinary urea, fecal N, and microbial purine flow were calculated for the last week of each period for each cow. Statistical analyses of mean data were performed using Proc Glimmix of SAS (Version 9.2, SAS Institute Inc., Cary, NC). Fixed effects were period, RUP concentration, RDP concentration, and the interactions of RUP and RDP concentration. Animal was included as a random effect. Unless otherwise stated, significance was declared at $P < 0.05$. All results were reported as least square means.

## RESULTS AND DISCUSSION

Observed DMI, CP, NDF, ADF, NFC, and fat were used to estimate $NE_L$, MP, RDP, and RUP supplied in dietary treatments (Table 4. 2) using the NRC model (2001). Measured CP contents of the High-RUP/High-RDP, High-RUP/Low-RDP, Low-RUP/High-RDP, and Low-RUP/Low-RDP treatment diets were 17.1, 16.6, 16.4, and 15.6% of diet DM, respectively which was slightly greater than the formulated concentrations of 16.5, 15.75, and 15.0% CP. The protein solubility (% of CP), starch, and NFC contents of diets containing low RDP were less than formulated values, whereas

NDF content was greater. The ADF and lignin content of the diet containing sub-NRC levels of RUP and RDP were greater than formulated values. On average, the NDF, ADF, NFC, and fat contents of the experimental diets differed by 1.45 ± 0.98, 0.93 ± 0.75, 4.68 ± 1.06, and 0.56 ± 0.22 % of DM, respectively, from the formulated values. Predicted $NE_L$ of experimental diets did not differ from targeted values and did not vary by diet (Table 4. 2). Although diets were higher in protein than formulated, predicted RDP and RUP supplies were still below NRC requirements for the Low-RUP/Low-RDP diet.

Results of the *in situ* analyses for CP are presented in Table 4. 3. The NRC model adequately fit the observed ruminal CP degradation of ground dry corn grain, soybean meal, protected soybean meal, and soybean hulls as demonstrated in Figure 4. 1. Although data are not shown, the NRC model also adequately fit the observed ruminal CP degradation of alfalfa hay and mixed alfalfa and grass silage. The NRC model, however, did not adequately fit the observed ruminal CP degradation of corn silage, and therefore values predicted by the NRC model may not reflect true protein solubility and degradation.

The fraction escaping at time 0 (fraction A) was greater than NRC values for ground dry corn grain, soybean meal, and protected soybean meal while observed values for corn silage, alfalfa hay, mixed alfalfa and grass silage, and soybean hulls were less than NRC values. Additionally, the insoluble, potentially degradable fraction (fraction B) was also greater than NRC values for corn silage, mixed alfalfa and grass silage, and alfalfa hay while observed values for ground dry corn grain, soybean meal, soybean hulls, and protected soybean meal were less than NRC values.

The degradation rates of corn silage, mixed alfalfa and grass silage, and alfalfa hay were 8.18 ± 15.4, 4.17 ± 5.15, and 11.7 ± 28.2 %/h as compared to NRC values of 4.4, 10.6, and 17.8 %/h. The degradation rates for ground dry corn grain, soybean meal, and soybean hulls were 6.67 ± 6.51, 10.8 ± 28.4, and 7.50 ± 6.22 %/h as compared to NRC values of 4.9, 7.5, and 6.2%/h. The degradation rate for protected soybean meal was 1.67 ± 3.89 %/h, which is similar to the reported NRC value of 1.7 %/h. The rate of disappearance of protected soybean meal was less than that of soybean meal as expected given the processing of that ingredient.

Least square means for DMI, nutrient intake, and total tract apparent digestibility of nutrients are presented in Table 4. 4. The interaction of RUP and RDP had a significant effect on lignin and fat intake (Table 4. 4) but had no additional effects on any other nutrient intakes or apparent digestibilities. Although there was no effect of RDP and RUP interaction, animals fed the High-RUP/Low-RDP diet had a higher DMI as compared to animals fed the low RUP diets, which was not expected as low RDP diets often cause a drop in DMI (Table 4. 4). The change in DMI may be due to lower ADF and lignin content in diets containing low RDP (Table 4. 2) as compared to diets low in RUP content. As a result of diet composition, RUP in the diet had an observed affect on DMI ($P = 0.02$), indicating that MP supply and RUP degradability may be major determinants of feed intake and possibly energy requirements in lactating dairy cattle. Our observations contrast with the results of Santos et al. (1998) who reported no change in DMI or N intake when SBM was replaced by high RUP sources. However, Robinson et al. (1991) observed no change in DMI when mid-lactation cows were fed sub-NRC levels of RUP in the diet, indicating that an additional unknown factor may have

contributed to the reduction in DMI observed in the present study for animals fed diets low in RUP.

Neither reduced RDP, reduced RUP, nor the combination of reduced RDP and RUP caused a depression of microbial protein synthesis (Table 4. 5) from the rumen indicating that microbial growth was not affected. The lack of change in microbial flow does not explain the observed effects on DMI. Cyriac et al. (2008) observed reduced DMI with diets containing 7.6% RDP, which is consistent with prior observations (Allen, 2000). Reduced DMI associated with low RDP diets may be caused by reduced ruminal ammonia concentrations resulting in depressed fiber degradation (Firkins et al., 1986). The reduction of RDP in the diet did not significantly affect total tract apparent digestibility of fiber, which does not support an effect of dietary treatment on rumen function. In contrast, RDP contents as low as 7.4 and 6.8% (DM basis) have not affected DMI (Gressley and Armentano, 2007, Kalscheur et al., 2006, Reynal and Broderick, 2005). Results from the present study indicate that RDP supplied in diets was adequate to prevent depressed ruminal ammonia concentrations and fiber degradation at RDP concentrations less than that recommended by the NRC (2001). The reduction in DMI may have been the result of higher fiber content (Table 4. 2) or lower palatability of the low RUP diets.

Due to the overall depression in dry matter intake N, INDF, lignin, starch, non-fiber carbohydrate (NFC), and ash intakes were significantly reduced for animals fed the low RUP diets (Table 4. 4). Low dietary concentrations of RDP resulted in a significant increase of ADF, NDF, INDF, and ash intakes (Table 4. 2). Given that RDP concentration did not have a significant affect on DMI, the increase of nutrient intake observed must be due to increased nutrient composition of the low RDP diets. The

interaction of RUP and RDP concentrations had a significant effect on lignin and fat intake, which may also be due to observed variation in nutrient composition for diets low in RDP.

Total tract apparent digestibilities of DM, N, ADF, and NDF were significantly increased for animals fed diets low in RUP content (Table 4. 4), whereas the concentration RDP and the interaction of RUP and RDP concentration had no effect. Feed fermentability determines the minimum concentration of ruminal ammonia required to maximize digestion (Erdman et al., 1986). The low RUP diets had numerically higher N digestibility, suggesting that supplied RDP and N return to the rumen were sufficient to maintain rumen function and maximize digestion as less DM was consumed. Robinson et al. (1985) observed that lactating dairy cattle that consumed low levels of DMI resulted in increased apparent digestibility of OM, N, NDF and ADF ($P$ <0.05), supporting the results of the present study. Conversely, Colucci et al. (1982) observed that reduced DMI only increased the apparent digestibility of ADF and cellulose, while increased DMI increased apparent digestibility of DM, NDF, hemicellulose, N, and energy. Thus, reduced DMI may only partially explain the observed differences in apparent digestibility demonstrated in the present trial.

Least square means for fecal composition, fecal excretion, urine excretion, and microbial protein synthesis are presented in Table 4. 5. Concentration of RUP in the diet significantly affected fecal composition and excretion of nutrients. Fecal output (kd/g) mirrored DMI. Additionally, low concentrations of RUP in the diet significantly reduced N output (kg/d; P = 0.0005) and numerically reduced fecal N composition (%). Daily fecal N output was comparable to a study performed by Davidson et al. (2003) that used similar diets varying in RUP content.

Treatment did not affect urine volume. Urinary urea N and total N excretion were significantly reduced in animals fed diets low in RUP, which reflects the reduction in N intake. Urinary urea N excretion was comparable with observations reported by Colmenero and Broderick (2006a), but lower than those reported by Broderick and Clayton (1997) and Reynal and Broderick (2005) as those studies examined diets with higher levels of RDP and RUP. Total urinary N excretion was consistent with prior observations that used similar diets (Davidson et al., 2003, Galo et al., 2003, Groff and Wu, 2005). Lactating cows fed diets low in RUP excreted less urinary N, which indicates increased efficiency in utilization of dietary protein (Table 4. 6) and lowered ruminal ammonia production as N recycled to the rumen is inversely related to N consumed (Reynolds and Kristensen, 2008).

Consumed dietary nucleic acids and nucleic acids synthesized by rumen bacteria are utilized by rumen microbes to produce microbial nucleic acids (Chen, 1992). Nucleic acids that leave the rumen are hydrolyzed and absorbed as purine nucleosides and free bases in the small intestine (Chen, 1992). Absorbed purines are subsequently catabolized by the kidney and excreted as the purine derivatives (PD), hypoxanthine, xanthine, uric acid, and allantoin (Perez et al., 1996). Allantoin and uric acid are the only PD present in cattle urine due to high xanthine oxidase activity in blood and tissues (Chen, 1992) thus excretion of urinary PD are directly related to microbial purine flow from the rumen (Chen, 1992, Chen et al., 1990). Neither dietary RUP nor RDP significantly affected uric acid (mmol/d), allantoin (mmol/d), total PD (mmol/d), or derived microbial N flows (Table 4. 5). Urinary output of uric acid, allantoin, and microbial N flow were consistent with data reported previously that use similar diets (Colmenero and Broderick, 2006a, Reynal and Broderick, 2005). These results indicate that microbial protein synthesis and

fiber degradation were not affected by levels of RUP and RDP suggesting that the NRC (2001) model may overestimate RUP and RDP requirements for lactating dairy cattle.

Milk production (kg/d), milk component yields (kg/d), and milk composition (%) were not significantly affected by treatment (Table 4. 6). Consistent with previously performed studies with similar diets (Cyriac et al., 2008, Rius et al., 2010), the reduction in RUP and RDP below NRC recommendations did not result in the loss of milk production. It is possible that the period length in the present study was too short to observe the extent of dietary responses in animals. However, the effect of period length is unlikely as diets low in RDP content rapidly compromise fiber digestion, and there was no apparent effect of RDP observed on rumen function in the present study. The lack of reduction in milk yield may have been due to the buffering effect of energy and N stores of the body, however observed BW change and N balance were not significantly affected by dietary treatments, indicating that animals received sufficient energy N and did not rely on body reserves to compensate for reduced N supplied in the diet (Table 4. 6).

The interaction of dietary RUP and RDP concentration trended to effect milk fat percent, while the individual concentrations of RUP and RDP had no effect (Table 4. 6). As a result, milk fat percent trended to be reduced when animals were fed the High-RUP/Low-RDP diet, and increased when animals were fed diets low in RUP. The trend for higher milk fat percent for animals fed the Low-RUP/Low-RDP diet may be due to the increased digestibility of NDF of diets low in RUP, while increased starch intake for animals fed the High-RUP/Low-RDP diet may have caused the observed reduction in milk fat percent. In a meta-analysis performed by Oba and Allen (1999), it was reported that a one-unit increase in NDF digestibility was associated with a 0.17 kg increase in DMI and a 0.25 kg increase in 4% fat-corrected milk. In contrast to the results reported

by Oba and Allen, the present study observed a decrease in DMI with increased NDF digestibility (Table 4. 4). Palmquist et al. (1993) observed that increased starch intake depressed milk fat percent, which is similar to results observed in the present study for animals fed diets high in RUP. Given that animals fed diets low in RUP had reduced DMI, reduced starch intake, and increased fiber digestion indicates that the increased milk fat percent observed for animals fed diets low in RUP content may be due to increased fiber digestion without milk fat depression normally observed with high intakes of starch.

Least square means of milk somatic cell scores (SCS) ranged between 4.44 and 4.91 (Table 4. 6), and it was observed that RUP concentration ($P = 0.002$) and the interaction of RUP and RDP concentration ($P = 0.03$) significantly reduced SCS in animals fed diets low in RUP content. All animals, except for 3, were >60 days postpartum indicating they were beyond the period post-calving when their immune systems are most severely suppressed and vulnerable to infectious diseases (Goff, 2006). Additionally, N balance was not affected by diet (Table 4. 6) indicating that negative protein balance amongst animals on low RUP diets does not explain differences observed in SCS. Milk urea N concentration ($P = 0.0009$) was significantly reduced while N efficiency percent ($P = 0.001$) was increased for animals fed diets low in RUP content (Table 4. 6). The reduction in MUN excretion as a result of decreased RUP content is consistent with prior observations that used diets varying in RUP content (Rius et al., 2010). Observed decreases in MUN concentration reflects reduced levels of MP and microbial protein being absorbed and catabolized by the small intestine and the liver, respectively. Reduced absorption of protein has been observed to result in less amino acid catabolism and therefore decreased urea production, recycling, and equilibrium with

bodily fluids (Broderick and Clayton, 1997). Animals fed diets containing RUP concentrations below NRC (2001) recommendations had significantly reduced BUN concentrations, as reflected by MUN, which did not result in compromised milk production or milk protein yield, indicating that protein requirements were maintained while simultaneously reducing nitrogen excretion from lactating dairy cattle.

## CONCLUSIONS

In this experiment, diets containing sub-NRC concentrations of RUP and RDP did not depress rumen fermentation or rumen microbial activity in lactating dairy cattle. However, diets containing low levels of RUP also contained higher composition of ADF and lignin, which resulted in reduced DMI, reduced nutrient intake, and increased apparent nutrient digestibility. As a result, animals fed diets low in RUP content consumed less dietary N and excreted significantly less fecal N, urinary urea-N, milk N, and milk urea-N which led to increased milk-N efficiency. Animals maintained rumen function, and had no loss in milk production when fed diets containing sub-NRC concentrations of both RUP and RDP, suggesting that the NRC overestimates requirements for RDP and MP. Dairy farmers can reduce feed costs associated with protein, ammonia emissions, while maximizing cow N efficiency through the reduction of RUP and RDP in herd rations.

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Figure 4. 1 In situ crude protein degradability of ground dry corn grain (—, ◇), soybean meal (– –, △), protected soybean meal (– · –, ○), and soybean hulls (---, □) in the rumen. The lines represent predicted values and the symbols the observed values.

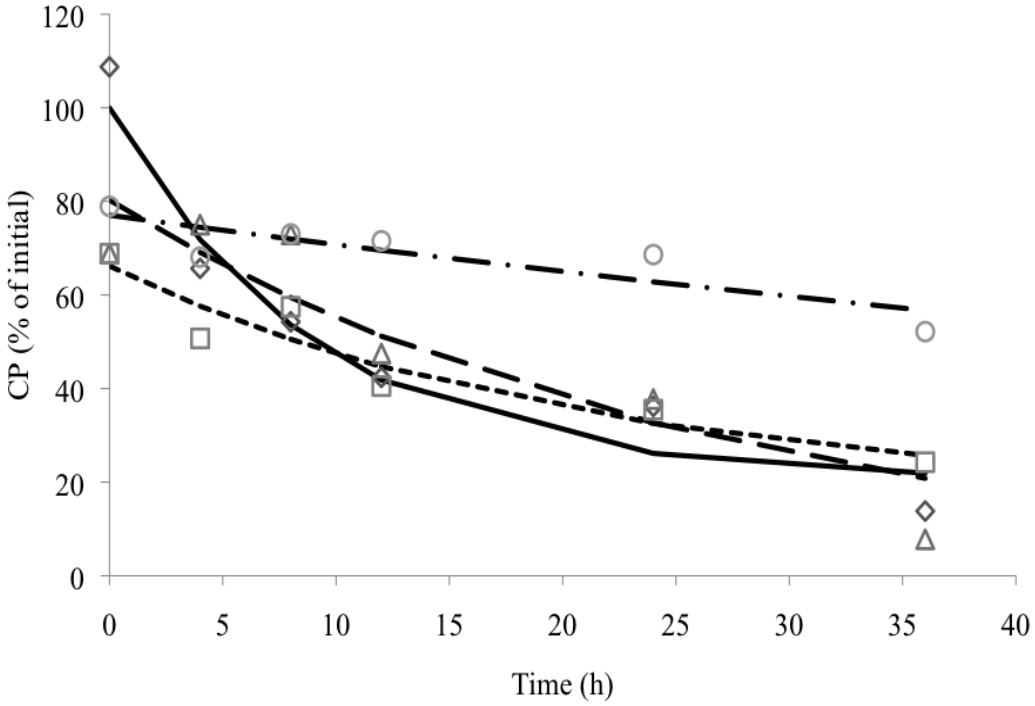

Table 4. 1 Composition and ingredient inclusion of diets fed in the trial.

| | Treatment, % of DM[1] | | | |
|---|---|---|---|---|
| Item | H-H | H-L | L-H | L-L |
| *Ingredient (% of DM)* | | | | |
| Corn silage | 26.8 | 26.8 | 26.8 | 26.7 |
| Mixed alfalfa and grass silage | 11.2 | 11.2 | 11.2 | 11.1 |
| Alfalfa hay | 8.92 | 8.93 | 8.92 | 8.91 |
| Ground dry corn grain | 13.6 | 12.1 | 14.4 | 13.1 |
| Soybean meal, solvent-extracted | 2.23 | 0.00 | 2.23 | 0.00 |
| Protected soybean meal[2] | 5.35 | 8.05 | 3.25 | 5.35 |
| Soybean hulls | 29.7 | 30.9 | 30.7 | 32.3 |
| Urea | 0.27 | 0.00 | 0.33 | 0.09 |
| Tallow | 0.71 | 0.80 | 1.03 | 1.02 |
| Calcium carbonate | 0.13 | 0.13 | 0.13 | 0.13 |
| Dicalcium Phosphate | 0.54 | 0.54 | 0.49 | 0.53 |
| Sodium bicarbonate | 0.20 | 0.20 | 0.20 | 0.20 |
| Salt | 0.42 | 0.42 | 0.42 | 0.42 |
| 0.06% Selenium premix | 0.03 | 0.03 | 0.03 | 0.03 |
| Trace premix rumin | 0.01 | 0.01 | 0.01 | 0.01 |
| Vitamin ADE mix[3] | 0.003 | 0.003 | 0.003 | 0.003 |
| Vitamin E (60000 IU) | 0.002 | 0.002 | 0.002 | 0.002 |

[1]Treatments: H-H = High-RUP/High-RDP, H-L = High-RUP/Low-RDP, L-H = Low-RUP/High-RDP, and L-L = Low-RUP/Low-RDP.
[2]Non-enzymatic Browned SBM, West Central Cooperative, Ralston, IA.
[3]Contained (% DM) Vitamin A, 26,485 KIU/kg; Vitamin D, 8,828 KIU/kg; Vitamin E, 44,141 mg/kg.

Table 4. 2 Observed and predicted nutrients supplied in diets as determined from the NRC (2001) model.

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | H-H | H-L | L-H | L-L |
| *Observed nutrients (% of DM)*[2] | | | | |
| DM | 62.5 | 63.9 | 64.9 | 66.0 |
| CP | 17.1 | 16.6 | 16.4 | 15.6 |
| Protein solubility, % of CP | 32.4 | 25.5 | 29.3 | 26.8 |
| Starch | 20.9 | 19.7 | 20.3 | 18.3 |
| NFC | 35.1 | 33.3 | 33.9 | 32.6 |
| NDF | 38.4 | 40.3 | 39.9 | 41.9 |
| ADF | 26.6 | 27.8 | 28.5 | 29.7 |
| INDF | 9.17 | 9.33 | 8.53 | 8.83 |
| Lignin | 4.19 | 3.68 | 3.94 | 4.29 |
| Fat | 4.12 | 4.52 | 4.61 | 4.30 |
| Ash | 6.14 | 6.19 | 6.11 | 6.31 |
| *Predicted nutrients (% of DM)*[3] | | | | |
| $NE_L$, Mcal/kg DM | 1.56 | 1.56 | 1.56 | 1.56 |
| RDP required, g/d | 2575 | 2620 | 2387 | 2311 |
| RDP supplied, g/d | 2596 | 2294 | 2390 | 2059 |
| RDP balance, g/d | 21.0 | -326 | 3.00 | -252 |
| RUP required, g/d | 1273 | 1489 | 1346 | 1553 |
| RUP supplied, g/d | 1768 | 1977 | 1412 | 1437 |
| RUP balance, g/d | 494 | 488 | 66.0 | -116 |
| MP required, g/d | 2564 | 2600 | 2488 | 2477 |
| MP supplied, g/d[4] | 2966 | 3001 | 2540 | 2384 |
| MP balance, g/d | 402 | 401 | 53.0 | -93.0 |
| $NE_L$ allowable milk, kg/d | 44.3 | 45.4 | 40.2 | 38.5 |
| MP allowable milk, kg/d | 45.3 | 45.3 | 37.5 | 34.2 |

[1]Treatments: H-H = High-RUP/High-RDP, H-L = High-RUP/Low-RDP, L-H = Low-RUP/High-RDP, and L-L = Low-RUP/Low-RDP.
[2]Observed mean values calculated across all 4 periods.
[3]Calculated using the NRC model, observed ingredient composition, and mean DMI by treatment.
[4]Assumes microbial yields are compromised by either an RDP or RUP deficiency.

Table 4. 3 Crude Protein solubility and degradation results from in situ analyses.

| Item | A, [1] % | B, % | C, % | k,$h^{-1}$ |
|---|---|---|---|---|
| Corn silage | 37.2 | 58.4 | 4.37 | 0.08 |
| Mixed alfalfa and grass silage | 25.1 | 67.9 | 6.93 | 0.04 |
| Alfalfa hay | 34.9 | 58.5 | 6.53 | 0.12 |
| Ground dry corn grain | 24.9 | 65.1 | 10.0 | 0.07 |
| Soybean meal, solvent-extracted | 39.4 | 56.1 | 4.52 | 0.11 |
| Protected soybean meal[2] | 26.7 | 71.2 | 2.11 | 0.02 |
| Soybean hull | 18.8 | 68.2 | 12.9 | 0.08 |

[1]A = soluble, B = insoluble (100 – A – C), C = undegradable, k = degradation rate.
[2]Non-enzymatic Browned SBM, West Central Cooperative, Ralston, IA

Table 4. 4 Effect of dietary RUP and RDP concentration on DMI, nutrient intake, and total tract apparent digestibility of nutrients in dairy cows. Data are presented as least square means (n=134, all variables).

| | Treatment[1] | | | | P > F[2] | | |
|---|---|---|---|---|---|---|---|
| Item | H-H | H-L | L-H | L-L | RUP | RDP | RUP×RDP |
| Intake (kg/d) | | | | | | | |
| DM | 26.4 | 27.6 | 25.1 | 24.6 | 0.02 | 0.14 | 0.32 |
| Total N, g/d | 719 | 734 | 656 | 615 | <.0001 | 0.53 | 0.18 |
| ADF | 7.03 | 7.62 | 7.13 | 7.32 | 0.85 | 0.008 | 0.28 |
| NDF | 10.2 | 11.0 | 9.98 | 10.3 | 0.29 | 0.01 | 0.43 |
| INDF[3] | 2.42 | 2.63 | 2.14 | 2.18 | 0.0002 | 0.009 | 0.49 |
| Lignin | 1.09 | 1.00 | 0.99 | 1.04 | 0.01 | 0.04 | 0.002 |
| Starch | 5.55 | 5.43 | 5.09 | 4.54 | 0.0003 | 0.29 | 0.19 |
| NFC[4] | 9.25 | 9.23 | 8.52 | 8.10 | 0.002 | 0.97 | 0.57 |
| Fat | 1.09 | 1.26 | 1.15 | 1.06 | 0.68 | 0.0003 | <.0001 |
| Ash | 1.62 | 1.71 | 1.53 | 1.56 | 0.02 | 0.04 | 0.58 |
| Apparent digestibility (%) | | | | | | | |
| DM | 58.2 | 57.7 | 61.6 | 62.2 | 0.01 | 0.62 | 0.84 |
| Total N | 57.4 | 54.0 | 59.9 | 58.8 | 0.09 | 0.06 | 0.62 |
| ADF | 44.1 | 45.1 | 49.6 | 51.5 | 0.003 | 0.76 | 0.99 |
| NDF | 40.5 | 41.9 | 45.1 | 47.9 | 0.01 | 0.53 | 0.88 |

[1]Treatments: H-H = High-RUP/High-RDP, H-L = High-RUP/Low-RDP, L-H = Low-RUP/High-RDP, and L-L = Low-RUP/Low-RDP.
[2]Probability of a significant effect of dietary treatment, RUP concentration, RDP concentration, or the interaction of RUP and RDP concentration.
[3]INDF = Indigestible neutral detergent fiber.
[4]NFC = Non-fiber carbohydrate.

Table 4. 5 Effect of dietary RUP and RDP concentration on fecal and urine composition, fecal and urine N excretion, and microbial protein synthesis in dairy cows (least squares means; n=128, fecal data; n=134, all other variables).

| | Treatment[1] | | | | $P > F$[2] | | |
|---|---|---|---|---|---|---|---|
| Item | H-H | H-L | L-H | L-L | RUP | RDP | RUP×RDP |
| *Fecal composition (%)* | | | | | | | |
| DM | 15.7 | 15.8 | 15.4 | 15.3 | 0.07 | 0.43 | 0.72 |
| Total N | 2.79 | 2.89 | 2.75 | 2.72 | 0.19 | 0.10 | 0.22 |
| ADF | 35.9 | 35.9 | 37.4 | 38.2 | 0.003 | 0.95 | 0.61 |
| NDF | 55.3 | 54.9 | 56.9 | 57.8 | 0.005 | 0.57 | 0.45 |
| INDF | 22.7 | 22.8 | 22.8 | 23.5 | 0.75 | 0.81 | 0.74 |
| *Fecal excretion (kg/d)* | | | | | | | |
| DM | 11.0 | 11.6 | 9.80 | 9.28 | 0.001 | 0.24 | 0.32 |
| Total N, g/d | 308 | 336 | 267 | 250 | 0.0005 | 0.08 | 0.17 |
| ADF | 3.91 | 4.18 | 3.66 | 3.56 | 0.05 | 0.17 | 0.33 |
| NDF | 6.06 | 6.39 | 5.58 | 5.38 | 0.02 | 0.26 | 0.37 |
| *Urine excretion* | | | | | | | |
| Urine volume, L/d | 18.8 | 19.1 | 18.3 | 18.4 | 0.52 | 0.67 | 0.95 |
| Urea N, g/d | 117 | 108 | 93.8 | 80.1 | 0.001 | 0.20 | 0.97 |
| Total N, g/d | 324 | 348 | 330 | 297 | 0.76 | 0.52 | 0.10 |
| Uric acid, mmol/d[3] | 37.0 | 37.4 | 34.5 | 35.4 | 0.39 | 0.76 | 0.85 |
| Allantoin, mmol/d[3] | 435 | 430 | 411 | 434 | 0.42 | 0.86 | 0.44 |
| Total PD, mmol/d[3] | 472 | 468 | 445 | 469 | 0.39 | 0.84 | 0.45 |
| Microbial N flow, g/d[4] | 318 | 315 | 298 | 317 | 0.36 | 0.85 | 0.42 |

[1]Treatments: H-H = High-RUP/High-RDP, H-L = High-RUP/Low-RDP, L-H = Low-RUP/High-RDP, and L-L = Low-RUP/Low-RDP.
[2]Probability of a significant effect of dietary treatment, RUP concentration, RDP concentration, or the interaction of RUP and RDP concentration.
[3]Excretion of urinary purine derivatives.
[4]Estimated microbial N outflow from the rumen (based on urinary PD excretion).

Table 4. 6 Effect of dietary RUP and RDP concentration on milk yield, milk composition, milk N excretion, and N efficiency in dairy cows (least squares means; n=134, all variables).

| | Treatment[1] | | | | $P > F$[2] | | |
|---|---|---|---|---|---|---|---|
| Item | H-H | H-L | L-H | L-L | RUP | RDP | RUP×RDP |
| Milk, kg/d | 33.8 | 33.3 | 35.3 | 33.4 | 0.47 | 0.53 | 0.56 |
| Milk N, % | 0.49 | 0.50 | 0.50 | 0.49 | 0.85 | 0.22 | 0.12 |
| Yield, g/d | 168 | 166 | 174 | 164 | 0.61 | 0.60 | 0.50 |
| Milk protein, % | 3.16 | 3.19 | 3.16 | 3.14 | 0.85 | 0.22 | 0.12 |
| Yield, kg/d | 1.07 | 1.06 | 1.11 | 1.04 | 0.61 | 0.60 | 0.50 |
| Milk lactose, % | 4.64 | 4.66 | 4.67 | 4.69 | 0.36 | 0.65 | 0.80 |
| Yield, kg/d | 1.58 | 1.57 | 1.66 | 1.57 | 0.43 | 0.66 | 0.48 |
| Milk fat, % | 3.80 | 3.70 | 3.84 | 3.91 | 0.15 | 0.14 | 0.06 |
| Yield, kg/d | 1.28 | 1.25 | 1.35 | 1.29 | 0.27 | 0.37 | 0.74 |
| Milk SNF, %[3] | 8.64 | 8.68 | 8.68 | 8.72 | 0.24 | 0.28 | 0.88 |
| Yield, kg/d | 2.99 | 2.91 | 3.06 | 2.94 | 0.67 | 0.50 | 0.82 |
| MUN, mg/dl | 12.4 | 11.6 | 11.1 | 9.49 | 0.0009 | 0.06 | 0.44 |
| SCS, Log(SCC) | 4.91 | 4.63 | 4.44 | 4.59 | 0.002 | 0.24 | 0.03 |
| N balance, g/d[4] | -79.7 | -108 | -112 | -96.1 | 0.34 | 0.50 | 0.29 |
| N efficiency, %[5] | 23.8 | 23.7 | 27.7 | 27.7 | 0.001 | 0.63 | 0.80 |
| BW, kg | 522 | 531 | 542 | 520 | 0.03 | 0.97 | 0.004 |
| BW change, kg | -10.7 | -11.3 | -7.07 | -12.7 | 0.43 | 0.52 | 0.33 |

[1]Treatments: H-H = High-RUP/High-RDP, H-L = High-RUP/Low-RDP, L-H = Low-RUP/High-RDP, and L-L = Low-RUP/Low-RDP.
[2]Probability of a significant effect of dietary treatment, RUP concentration, RDP concentration, or the interaction of RUP and RDP concentration.
[3]Milk SNF = Milk solids non-fat.
[4]N balance = intake N – (milk N + urine N + fecal N).
[5]N efficiency = (milk N ÷ intake N) × 100

## CHAPTER 5: General Conclusions

# CONCLUSIONS

A heightened demand for animal products priced affordably has resulted in highly specialized, densely stocked animal operations that produce and emit excess N into the environment. The United States government has enacted several laws and regulations, such as the Clean Water Act and the Clean Air Act, to address current environmental problems associated with agricultural N pollution and to limit future N loading from livestock. The necessity to produce animal products for a growing population and limit environmental damage has led to extensive strategies to maximize animal N efficiency without affecting production.

Milk urea N is correlated with dietary N content, N intake, N balance, and N excretion and is therefore commonly used by dairy producers and DHI programs to monitor protein-feeding management. In addition to dietary N, several environmental and genetic factors are known to cause deviations in MUN from expected value, which poses a problem if it is used as a protein feeding monitor. Given that MUN is a highly heritable trait amongst dairy cattle, it is possible that cow and therefore herd variation may greatly influence observed MUN concentrations. Thus, the objective of the first study was to determine MUN variation associated with animal and herd.

Analyses of research data from two previous studies demonstrated that MUN concentrations could be predicted equally well from diet composition, milk yield, and milk components regardless of whether DMI was included in regression models and that

cow was a highly significant determinant of MUN. Field trial data supported the observation that cow was a significant determinant and herd trended to significance. Additionally, it was determined that a percentage unit change in dietary CP content resulted in a 1.1 mg/dl change in MUN. The observed cow effect indicates that adjusting dairy rations solely on CP content may result in compromised milk production if genetic variation exists amongst animals. Thus, the observed phenotypic variation in MUN is potentially the result of genetic differences amongst cows and must be considered when using MUN to adjust feeding management.

Several factors including urea synthesis, urea transporter abundance, or urea transporter activity could be the source of genetic variation amongst cows and thus the cause of the observed phenotypic variation in MUN. Urea synthesis is driven by protein catabolism, and thus is most likely not the source of variation amongst cows. Data from trials performed previously have not observed a correlation between urea transporter abundance and urea extraction by gastrointestinal (GIT) epithelial cells, indicating further investigation of urea transport activity. Differences in urea transport activity of the kidney or rumen wall epithelium could potentially be the source of variation among animals. Therefore, the second objective was to determine if urea transport into the digestive tract or urine was the source of animal variation in MUN.

Eight lactating dairy cattle with similar milk production, but varying MUN, were fed a common diet and assessed for N balance and urea kinetics over a 4 d period. Consistent with prediction models, MUN averaged 14.9 mg/dl for animals, but it ranged from 7.33 to 20.8. Gastrointestinal tract urea clearance rates were negatively associated with MUN ($P = 0.008$) and PUN ($P = 0.02$) concentrations, indicating that difference in MUN among animals was at least partially due to differences in clearance rates across the

GIT. Kidney urea clearance rates were not correlated with either MUN or PUN, and thus were not the source of variation in MUN. Differences in clearance rates may be due altered amounts of transporters (Vmax), altered transporter substrate affinity (Km), or transporter saturation. Given that there were no differences in dietary N intake, milk yield, urea excretion, or urea utilization by tissues, and that dietary protein was set to moderate levels, it is difficult to envision transporter saturation as being the contributor to the observed variation in GIT clearance rates. Thus either Vmax or Km must be varying among animals, indicating that variation in urea transport may predispose cows to a RDP deficiency if they are fed diets low in RDP and have poor urea transport into the GIT. The results from this trial can be used to sort cows by MUN concentration and potentially feed low MUN concentration cows with lower dietary RDP to improve urea transport efficiency to the rumen.

The results of the second study confirm that observed phenotypic variation in MUN is due at least partially to differences in GIT urea clearance, although other environmental and genetic factors may also be contributing. The significant influence of GIT urea clearance on MUN and PUN concentration suggests that requirements for ruminally degradable protein (RDP) might depend on differences in urea transport, as urea returned to the rumen is a source of RDP. Previous studies have demonstrated that RDP and RUP can be reduced independently below NRC requirements, with no effect on milk production, suggesting that NRC recommendations overestimate true protein needs. If affected by other dietary components, the exclusion of urea transport variation, such as GIT urea clearance, from NRC prediction equations may contribute to observed protein overestimation. Thus, the third objective was to determine the affect of sub-NRC levels

of RDP and RUP in diets varying in CP concentrations on intake, milk production and composition, and apparent N efficiency in lactating dairy cattle.

Varying levels of RUP and RDP had no effect on milk production, milk component yield, milk component percent, or rumen microbial activity. These results indicate that animals were provided sufficient amounts of protein to maintain rumen health and production, despite levels being below NRC requirements and sub-NRC requirement levels of both could be fed without compromising production. Diets containing low levels of RUP had significantly reduced N intake and excretion, which was the result of increased ADF and lignin composition of the diet. When fed diets containing sub-NRC levels of RDP and RUP, animals were able to maximize N utilization, maintained rumen function, and had no loss in milk production suggesting that the current NRC model may overestimate true protein requirements.

Progress toward a complete understanding of true protein and amino acid requirements of dairy cattle requires investigating all factors that affect an animal's ability to utilize and absorb nutrients. The results of the presented project provide evidence of potential genetic variation among dairy cattle due to differences in urea transport. Dairy cattle management can be improved to feed animals more effectively, which will result in reduced feed cost and N loss to the environment.

Future work in this field of research should include genetic studies to determine if the DNA makeup of urea transporters is different between cows with high or low MUN, as this would support the phenotypic variation observed in the present project. Additionally, a feeding trial with diets varying in concentrations of RUP and RDP should be performed to observe response of N efficiency between cows with intrinsically high or low MUN. The results of a feeding trial may support over-prediction of true protein

needs and demonstrate additional phenotypic variance in urea transport activity between animals.