EFFECT OF FEEDING CORN SILAGE BASED DIETS PREDICTED TO BE DEFICIENT IN EITHER RUMINAL NITROGEN OR METABOLIZABLE PROTEIN ON NITROGEN UTILIZATION AND EFFICIENCY OF USE IN LACTATING COWS

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Erin Beth Recktenwald

August 2007

ABSTRACT

Due to concern over nitrogen (N) emissions, this study attempted to evaluate dietary approaches to reduce N excretion by dairy cattle. Knowledge about potential N sources that were either unaccounted for or under-predicted by CPM Dairy and the Dairy NRC (2001) was used to formulate rations that were much lower in crude protein (CP) than typically fed to dairy cattle but would potentially not decrease production. Specifically, the three diets fed were predicted to have: (1) positive rumen N and metabolizable protein (MP) balances (Diet P) (2) negative MP balance and positive rumen N balance (Diet N), or (3) negative rumen N balance but positive MP balance (Diet T) as predicted by CPM Dairy version 3. The objective of this experiment was to determine whether, and to what extent, the decrease in predicted ruminally available N and MP supply would affect milk production.

Eighty-eight multiparous lactating Holstein cows (83 ± 20 DIM), were blocked by average daily milk yield to 50 DIM and parity and assigned to three diets differing in N content or predicted rumen degradability of the feed N. The diets were formulated with CPM Dairy V3 using library values for all feeds except corn silage where actual chemical, digestibility, and degradation rate values were determined and used. The diets (DM basis) consisted of approximately 50% corn silage, 2% wheat straw and 48% of a diet specific ingredient mix and were formulated for 22.2 of kg DMI. Actual diet CP levels were 16.7, 14.2 and 14.3% for Diets P, N and T, respectively. The predicted CPM Dairy rumen N balance at the formulated DMI was 29 and 27 g for Diets P and N and negative 39 g/d for Diet T, whereas the predicted MP balance was 263 and negative 145 and 91 g/d for Diets P, N and T, respectively. Monensin was included in the diets at a formulated intake of approximately 300 mg per cow per d and somatotropin was administered per label.

Actual DMI for cattle fed these treatments were 25.7, 25.5 and 24.2 kg/d for Diets P, N and T, respectively and were significantly lower for Diet T. Actual milk yield was 45.0, 42.6 and 43.3 kg/d and 3.5% FCM was 38.1, 36.5, and 36.4 kg/d for cows fed Diets P, N and T, respectively and was significantly lower for cows fed Diets N and T. Milk protein percent was not affected by diet; however, milk protein yield was significantly greater for cows fed Diet P due to the difference in milk yield. Plasma urea N concentrations were 11.31, 8.40 and 7.13 mg/dl for cows fed diets P, N and T, respectively and were different and paralleled the rumen ammonia levels of 8.32, 6.58 and 5.84 mg/dl. Milk fat depression (MFD) was observed in all cows and was not affected by treatment, and the average milk fat levels were 2.67, 2.68 and 2.54% for diets P, N and T, respectively. To determine if monensin was partially responsible for the MFD, monensin was removed from the diets of approximately half of the cows on treatment once they had finished the experimental period. Removal of monensin resulted in a 30% increase in milk fat percent, and milk protein content was not affected. Calculated milk N:intake N ratios for the three treatments were 0.31, 0.33 and 0.36 for Diets P, N and T respectively.

The results of this study suggest that more productive N is available than currently predicted by either CPM Dairy and the Dairy NRC (2001). Understanding where these differences exist would allow for feeding less CP to dairy cattle and decreasing N emissions to the environment. It may also be a profitable strategy for dairy farmers, as they would be able to reduce their purchase of costly protein feeds, but that was not demonstrated in this study – primarily due to the severe milk fat depression that decreased the economic value of milk. However, ration cost was not a concern for this experiment, and that aspect can be considered when implementing feeding strategies stemming from this research.

BIOGRAPHICAL SKETCH

Erin Beth Peterson was born in the lovely town of Frederic, WI. Her parents, Warren and Joan, raised her with a good sense of morals and common sense and always encouraged her to do well in school, which was never a problem since she enjoyed it anyway. Her older brother, Bryan, and her spent much of their childhood exploring the woods around home, and she carries this endearment to the outdoors to this day. She spent much time with her grandparents in Atlas, WI, and there are plenty of pictures to prove it. She is thankful to have been so close to relatives and able to develop those ties. Erin spent much of her time with her horses, Bint and Major, riding them through the countryside and, later in life, showing them for 4-H projects. Her enjoyment of working with large animals probably stems from this experience. She gained a lot of friends through the horse project and really enjoyed the times at Blake's house. She also loves music and playing sports.

Erin went to UW-River Falls to major in Animal Science. She initially planned to go into veterinary work, but after following around large animal vets, decided to stay in academia. She was highly involved in the InterVarsity Christian Fellowship group on campus, but also worked hard at classes and had fun being an organic chemistry teaching assistant. She decided to go to Cornell University after grad school, so packed up her things and headed East in the fall of 2003.

At Cornell, Erin had a lot of fun trying to settle on a research topic, but eventually started working on nitrogen metabolism. She also discovered the Graduate Christian Fellowship, gained a lot of good friends, and spent many late nights discussing nearly every topic under the sun. During this time, she met Geoff Recktenwald, who she came to admire as a thoughtful, respectable, enjoyable person. They were married in Oct. of 2006, and have had a wonderful beginning to their lives together.

To my parents, Warren and Joan, and my brother Bryan.

ACKNOWLEDGEMENTS

My first acknowledgement must go to the One who is continuously working in my life. Thank you to my Lord and Savior Jesus Christ for allowing me to do all things and to share hope.

I also thank my parents for such a fabulous childhood in the woods of Wisconsin and for teaching me well. You have given me a lot of wisdom that will continue to help me in all I do. Thanks Bryan for being such a neat brother. You are exactly what I need in a sibling. I thank my grandparents, David and Edith, for being the way they are, two nice, generous, enjoyable people to be around. I also thank my grandma, Cleithra, and step grandpa, Howard, for the fun times I spent with you and for being there for me. Thanks to the cousins, Caleb, Laura, and Kyle for the fun days on the farm and afterward. To Miriam, for having the patience to put up with all of us kids. To Mark, for being the neat uncle.

I'd also like to thank Jason and Tina Hull, for doing such a good job at UW-River Falls InterVarsity. Also, to all the friends from there, thanks for the wonderful time. To all the Ithaca friends, thanks for your understanding and generosity with time and friendship. Thank you to Geoff for your love and for all the support and encouragement you give. Thanks Angela for being a fabulous roommate. Thanks Steve for the interesting experiences at the old apartment, and for teaching us how to play bridge. Thanks Ray for your intensity and your challenge to study the Word. Thanks Jen for being in all those studies together, and for your patient and fun nature. Thank you Steve Felker for being a fantastic pastor and someone I can always ask tough questions to and get a straight answer.

A big thanks to Mike for being my advisor through this process. It's been a long process, but I've learned a lot and had a good time. Also, thanks to Debbie for putting

up with me in the lab; that's certainly enough in itself. Thanks Jimmy for all the encouraging words about being a Christian and from Wisconsin. Lots of gratitude toward the farm crew at the T&R, particularly toward Gladys for all her help. Also, thanks to Will for midnight milk sampling and to Tiberio, Isaac, Justine, and Marissa for all their assistance. You were all a great crew.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fiber
ADIN	Acid detergent indigestible fiber
AOAC	Association of Official Analytical Chemists
BCS	Body condition score
BCVFA	Branched chain volatile fatty acids
bST	Bovine somatotropin
BW	Body weight
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
CPM	Cornell Penn Miner
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECM	Energy corrected milk
EMPS	Efficiency of microbial protein synthesis
FCM	Fat corrected milk
FOM	Fermentable organic matter
GIT	Gastrointestinal tract
HPLC	High performance liquid chromatography
IOFC	Income over feed cost
Lys	Lysine
ME	Metabolizable energy
Met	Methionine

MFD	Milk fat depression
MP	Metabolizable protein
MUN	Milk urea nitrogen
N	Nitrogen
NAN	Non-ammonia nitrogen
NDF	Neutral detergent fiber
NDIN	Neutral detergent indigestible nitrogen
NH_3	Ammonia
NPN	Non-protein nitrogen
NRC	National Research Council
NSC	Non-structural carbohydrate
OM	Organic matter
PUFA	Polyunsaturated fatty acid
PUN	Plasma urea nitrogen
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
SCC	Somatic cell count
TDN	Total digestible nutrient
TMR	Total mixed ration
VFA	Volatile fatty acid

CHAPTER ONE: LITERATURE REVIEW

Introduction

The topic of nitrogen (N) metabolism and efficiency has gained increasing attention in recent years due to environmental concern over N emissions. In the air, nitrogen oxides can aid in the formation of ozone and particulate matter, which can cause a variety of respiratory illnesses, contribute to the formation of acid rain, and stimulate global warming (National Academies Press, 2003). On land, N can leach into ground water and form nitrates, which are lethal if ingested in high doses (Di and Cameron, 2002).

Ruminants are able to convert large amounts of plant material that is indigestible by mammalian enzymes into usable energy through microbial activity in their rumen. This provides them with an ability to convert these substances into useful products. Particularly, it enables inorganic N in the form of ammonia (NH_3) to be transformed into amino acids (AA) and protein, when availability of energetic substrates is adequate (Hanigan et al., 1998).

The ratio of N output in milk to N input from feed, which is an estimation of gross N efficiency, is typically 0.20 to 0.35 (Jonker et al., 2002; Ipharraguerre and Clark, 2005; Groff and Wu, 2005; Broderick, 2003;Noftsger and St Pierre, 2003). In a review of literature data it was reported that on average, 0.35 of the N ingested is excreted in feces, 0.34 in urine, and 0.31 in milk (Lapierre and Lobley, 2001). There is approximately 15-20 kg of protein growth from a cow's first calf until it ceases growing after the fourth lactation. However, compared to 0.4 to 0.5 kg excreted N/d, or 120-150 kg N/305d lactation, this retention of approximately 0.5 to 0.8 kg N/lactation is negligible. The waste N, ~70% of intake N, is then distributed into the environment, which can lead to environmental and human health problems.

According to the NRC 2001, as the amount of crude protein (CP) in the diet of dairy cattle increases, the amount of milk produced increases until it reaches maximal milk yield at approximately 23% CP (Figure 1). Further increases in the amount of CP per diet DM results in decreases in milk yield. However, the amount of excess N (intake N minus milk N, in the case strictly focusing on N use for milk production) increases with corresponding CP increases (Figure 2). Traditionally it has been economically profitable to feed higher CP concentrations in order to achieve the highest possible milk production as the revenues from milk production outweighed the cost of protein feeds to achieve it. St-Pierre and Thraen (1999) averaged the prices of 23 different feed ingredients over 15 years then used the relationships between nutrient intake and milk production by the NRC 1989 and a variety of statistical procedures to account for variation in BW, MPP, efficiency of nutrient use by the animal, and DMI. Milk production was maximized at 18.6% CP, but N efficiency was maximized at 14.9% CP and income over feed costs was maximized at 18.0% CP. The authors stated that transitioning the feeding strategy of dairy cattle from maximal income over feed costs to maximum N efficiency (milk N:excreted N ratio) would reduce national N excretion by 142,000 tons, but would cost $1.35 billion per year. However, the relationship between N intake and milk yield used in this analysis was based on CP intake rather than RDP and RUP. If the latter were used, it may be possible to refine N intake in such a way to reduce it without any or less detrimental effects on milk production.

There are several ways to reduce N accumulation to the environment caused by ruminants either without compromising milk and meat production or reducing it to an acceptable level given the corresponding reduction in N excretion. The first is to simply add less synthetic or natural fertilizer to fields. The second is to deposit fertilizer in areas and during times where the plant uptake is more than or equal to the

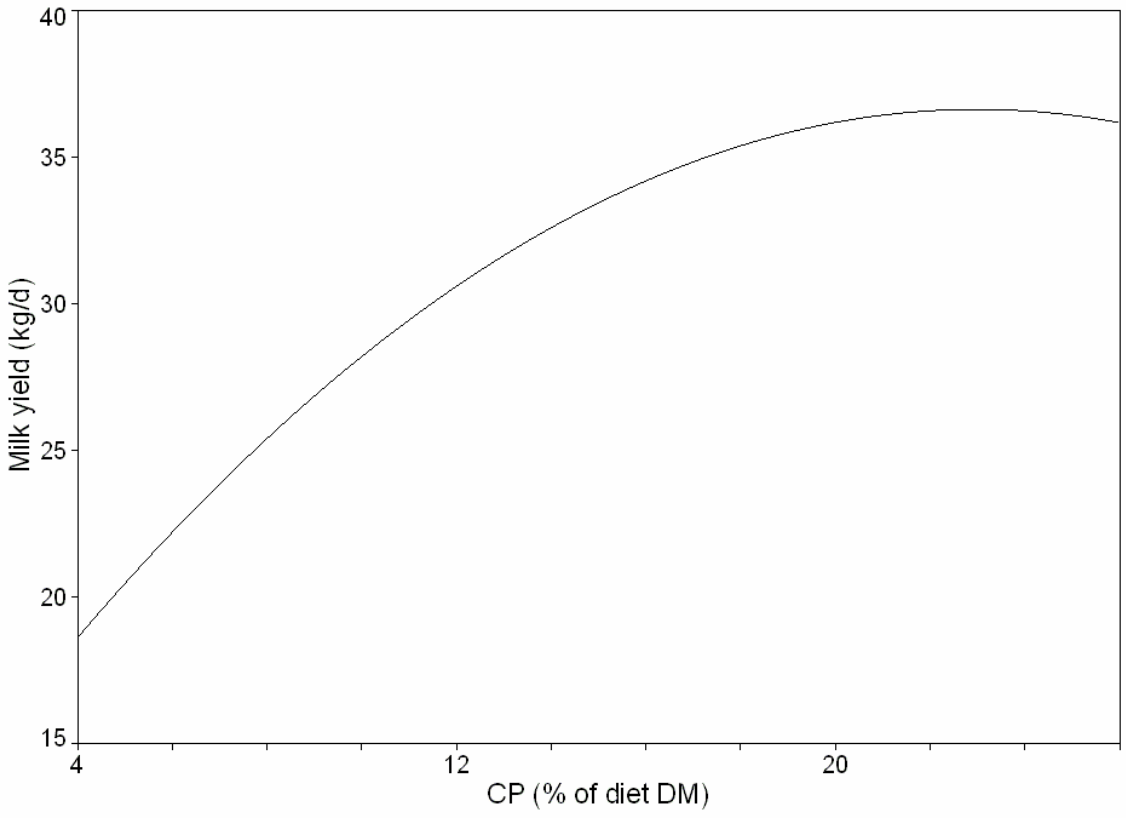

Figure 1. Milk yield (kg/d) versus CP (% of diet DM) using the equation: Milk yield (kg/d) = (0.8*DMI+2.3*CP-0.05*CP^2-9.8) from NRC (2001), with DMI at 25 kg/d.

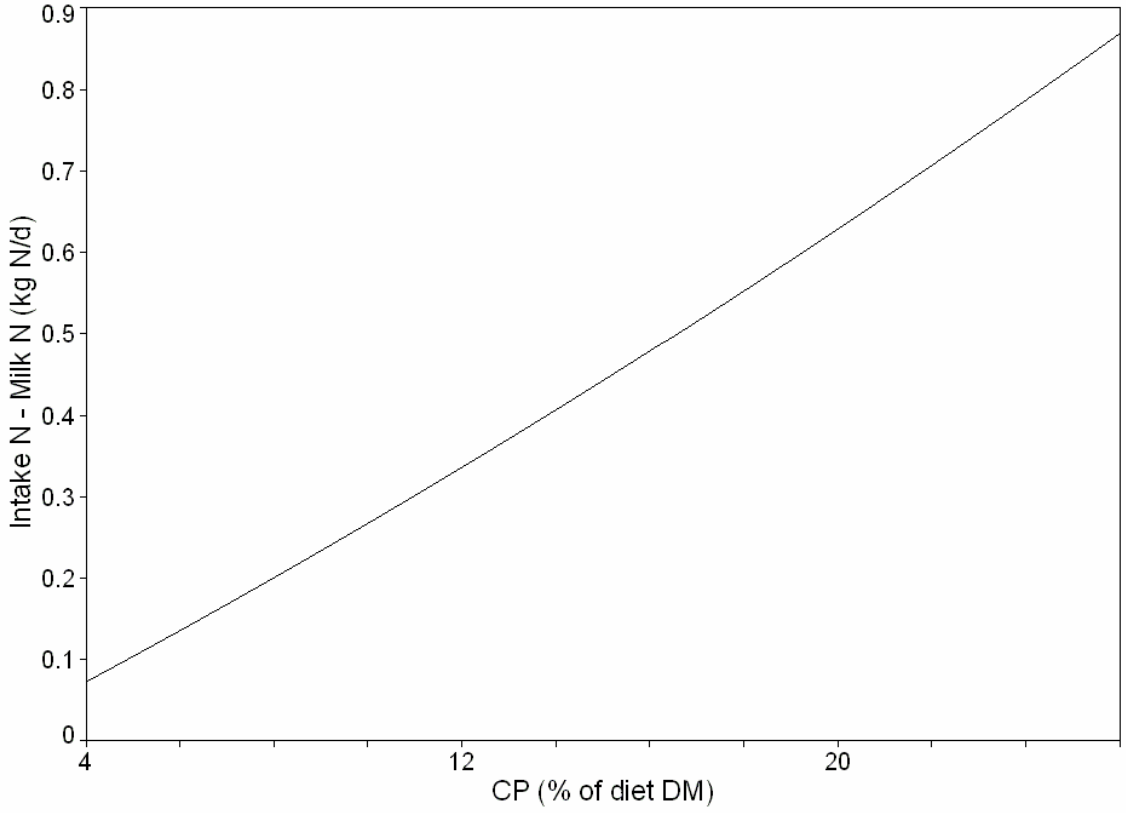

Figure 2. Nitrogen not used for milk production (Intake N – Milk N) in kg N/d versus CP (% of diet DM) using the equation: Milk yield (kg/d) = (0.8*DMI+2.3*CP-0.05*CP^2-9.8) from NRC (2001) for milk yield and assuming 2.95% milk protein and 25 kg DMI/d.

amount spread. The third is to manipulate the soil in such a way that a larger proportion of N is transferred to the atmosphere in the harmless form of molecular dinitrogen, which constitutes approximately 80% of atmospheric gases. The fourth is to find alternative uses for manure N so it will not be added to the environmental pool of N but will be used for a different purpose. The final approach is to reduce N inputs to cattle without any, or acceptably compromised, reductions in milk and meat production. This last approach will be the focus of this thesis.

Nitrogen Flows Within the Ruminant

The study of N usage within the cow has been the subject of numerous research reports and reviews (Cocimano and Leng, 1967; Oldham, 1984; Leng and Nolan, 1984; Bach et al., 2005; Lapierre et al., 2005; Nolan, 2005). Synthesis of this information is difficult, due to the volumes of information, biological variation, and differences in experimental technique. The generic N partitioning of the animal's intake N is summarized in Figure 3. An amount of N is consumed, which is put either into productive use, such as tissue growth or milk production, or is excreted in the feces, urine, or as gaseous N in the form of NH_3. Theoretically, these amounts should account for all of the N consumed, but in practice there is always an element of experimental error, in which the N is lost or not accounted for in the total scheme. For example, in growing animals, N balance studies will overestimate N intake and underestimate N excretion due to collection error, leading to variations of 20% or greater (Macrae et al., 1993; Diaz et al., 2001). This should be taken in consideration when using experimental data to infer accurate N flows, particularly the excretion of N, throughout the animal.

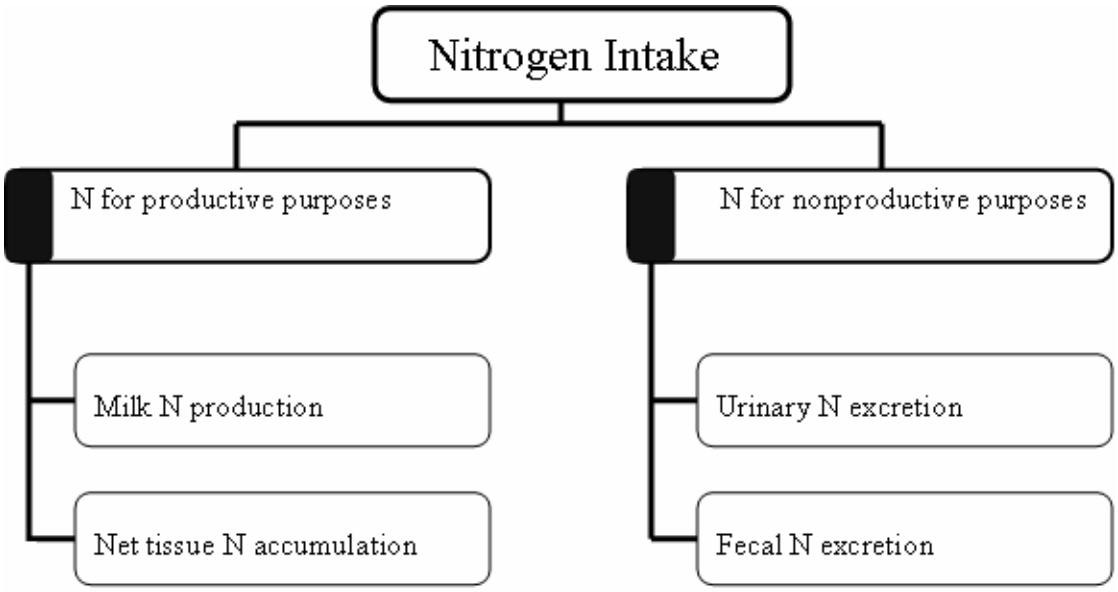

Figure 3. Generalized nitrogen partitioning scheme in a growing and/or lactating ruminant.

Ruminal Nitrogen Flows

There are several forms of N present in the rumen: ammonia (NH_3), amino acids (AA), peptides, and protein. Since the ruminal usage of N depends on the amount and form of N present, it would be beneficial to discuss the transactions among these forms. Rumen NH_3 can be produced in numerous ways. It can be the end product of hydrolysis and/or deamination of peptides and AA in the rumen, released by microbial death and lysis, or provided directly in the feed (Figure 4). Ammonia may exit the rumen through the following routes: rumen wall absorption into plasma, microbial utilization for growth, or passage with the rest of the digesta. Ruminal NH_3 concentrations are a function of the balance between these routes, which fluxuates throughout the day, with highest concentrations typically associated with the feeding pattern (Valkeners et al., 2006).

Peptides available for microbial utilization in the rumen originate from microbial death and lysis, from endogenous secretions, or directly from the feed (Figure 5). In some cases, these peptides may have been cleaved from a protein, such as those found in feed or microbial constituents. Flow of peptides out of the rumen pool may again be from microbial utilization or passage. It is generally assumed that peptides are not directly absorbed through the rumen wall (Lapierre et al., 2005).

The amount of total protein in the rumen is dependent on how much microbial protein was able to be synthesized, the amount of endogenous protein secretions into the rumen, and the amount present in the feed (Figure 6). This material leaves the pool by microbial use, deamination, or by passage from the rumen. Because of its close connection with peptides (i.e. proteolysis of protein forms smaller peptides) these two pools are considered to be quite similar and interconnected.

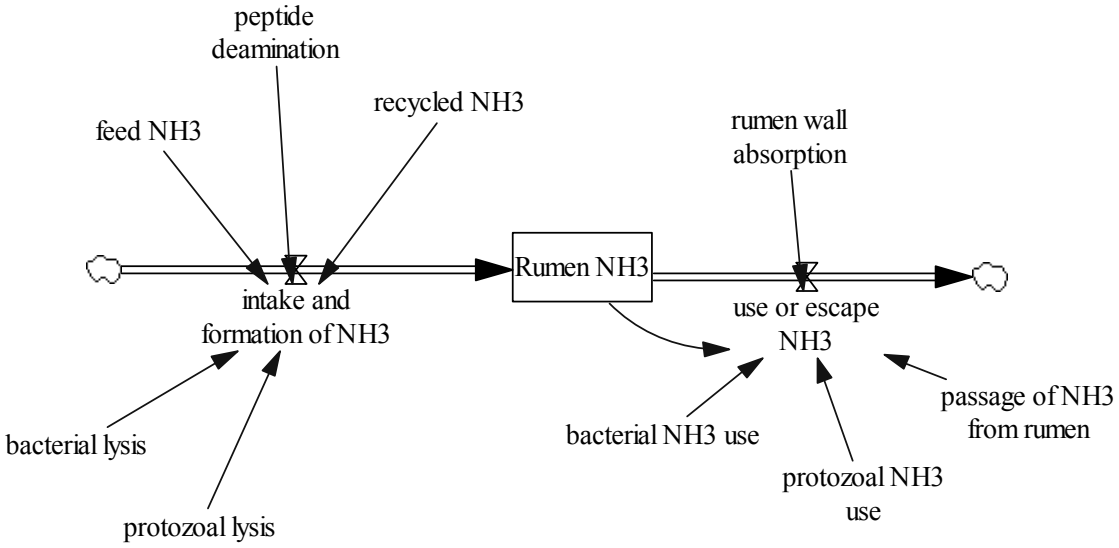

Figure 4. Ruminal ammonia transactions.

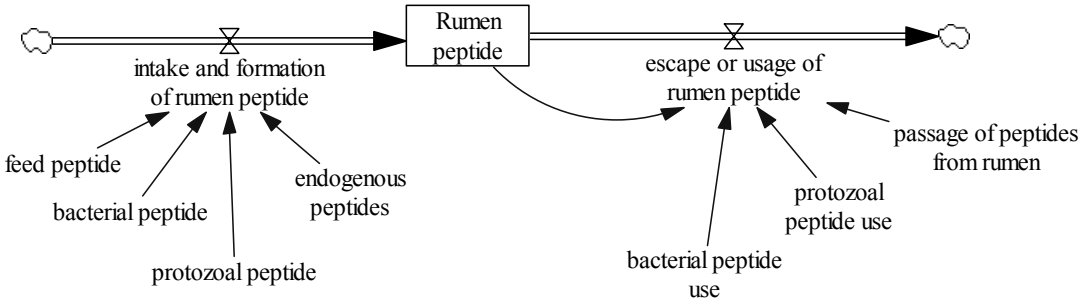

Figure 5. Ruminal peptide transactions.

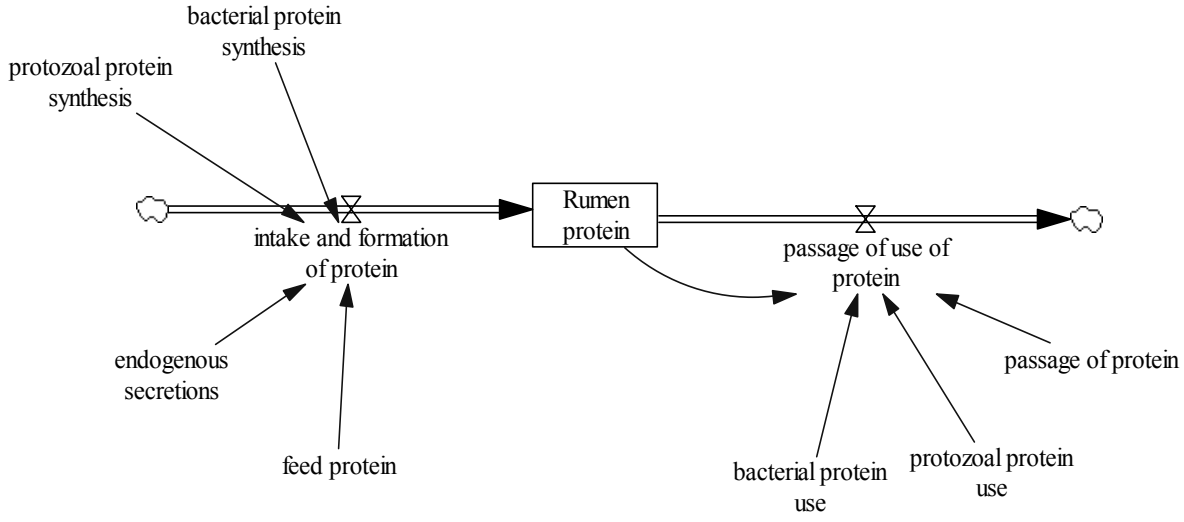

Figure 6. Ruminal protein transactions.

Microbial Nitrogen Supply

Ruminal microbes convert non-protein N (NPN) into protein forms. Microbial protein supplies a large proportion, 50-80%, of the absorbable protein to ruminants (Bach et al., 2005). Summarizing 152 treatment diets in dairy cows, it was reported that microbial N provided an average of 59% of the non-ammonia nitrogen (NAN) supply passing to the duodenum, with a range of 34 to 89% (Clark et al., 1992). Due to their favorable AA composition, they provide an excellent source of protein for the animal's productive needs . Therefore, it is of benefit to understand the process of and how to maximize microbial production in order to refine N management.

Microbial Ammonia Utilization

The contribution of NH_3 to microbial N varies according to the rumen's environmental conditions, such as the amount of available fermentable energy and the concentration of peptides and AA present. The measured amount of microbial N from NH_3 have ranged from 18 to 100% (Firkins et al., 1987; Salter et al., 1979; Wallace et al., 1999), but typically it averages around 80% (Bach et al., 2005). The Cornell Net Carbohydrate and Protein System (CNCPS) assumes structural carbohydrate fermenting bacteria to utilize only NH_3, while non-structural fermenting bacteria may also use peptides and AA (up to 2/3 of its N incorporation) (Russell et al., 1992). It is still unclear if this is consistent with all cellulolytic bacteria, as some experiments have suggested AA use by that population (Atasoglu et al., 2001), but it appears to be an adequate representation until more specific microbial requirements can be further elucidated. Under low NH_3 conditions it is possible that the rate of fermentation of nonstructural carbohydrates and the differences in growth rate of nonstructural bacteria versus structural bacteria could lead to an unfair competitive advantage for rumen NH_3 availability (Russell et al., 1992). Nonetheless, NH_3 is an important

microbial substrate, and knowledge of its inter- and extra-ruminal metabolism aids in understanding N excretion.

There has commonly been thought to be a minimal amount of NH_3 that must be present in the rumen environment for maximum microbial growth. This idea was pioneered by Satter and Slyter (1974) in which continuous culture fermentors were used to examine the impact of urea additions to the amount of tungstic acid precipitable N formed. They found no increases in tungstic acid precipitable N when ruminal NH_3 concentrations exceeded 2 mg NH_3N/dl, but recommended 5 mg/dl as a safe level. Consistent with these data, Clark et al., (1992) reported that passage of microbial N in dairy cows was correlated to NH_3 concentrations when in the range of 2-5 mg/dl, but the correlations were more highly related to organic matter totally digested if the NH_3 concentrations were above this level. This again suggests that ruminal NH_3 concentrations can be the limiting factor for microbial growth.

However, the reported NH_3 concentration at which maximum microbial growth is achieved is not consistent. In an original series of studies by Hoover and Stokes (1991), the NH_3 concentration at which no further increase resulted in increases in microbial growth depended on the amount of fermentable energy (Hoover and Stokes, 1991; Stokes et al., 1991b). More energy allows for more growth; therefore, the optimal NH_3 concentration varies by diet. Also, consideration should be given to the relative importance of NH_3 in the diet of specific microbes, and how the proportion of microbial protein growth from NH_3 varies with energetic substrate. Due to the rate of metabolism, not only will the non-structural fermenting bacteria not be able to fully utilize all N substrates, but the structural fermenting bacteria too will be starved of their primary N substrate. A possible example of this was observed in a study by (Ruiz et al., 2002), where in improvement in NDF digestibility occurred as ruminal NH_3 increased from 4.5 to 10 mg NH_3N/dl. Since ammonia concentration fluctuates

throughout the day (Valkeners et al., 2004; Oba and Allen, 2003), and the depression in digestion might be small, it is difficult to elucidate the impact of sub-optimal N supply on microbial function.

Peptides and Amino Acids in the Rumen

Peptides have been shown to have stimulatory effects on the growth of ruminal microbes (Chen et al., 1987; Russi et al., 2002; Wallace et al., 1999). They are converted into microbial protein with an efficiency of approximately 80% (Russell, 1983). This situation is believed to mainly occur in non-structural carbohydrate fermenting bacteria (Cruz Soto et al., 1994). In the CNCPS, NSC bacterial yield is increased up to 18.7% as the ratio of peptides to NSC plus peptides is increased up to 14% (Russell et al., 1992). Since peptides have been observed to accumulate in the rumen, up to 200 mg N/L (Eschenlauer et al., 2002), it is possible that their uptake can be a limiting step in peptide utilization (Chen et al., 1987). Therefore, not all peptides are metabolized, and they may also exit the rumen in significant amounts to play a role in protein supply in the intestines.

Microbial Protein Synthesis Efficiency

Microbial efficiency can be defined as grams of microbial N passing to the duodenum per kilogram of true ruminally degraded organic matter (Oba and Allen, 2003). Values of 12 to 54 g/kg have been reported (NRC, 2001). Some researchers have made general relational observations of microbial protein synthesis to other variables, but these correlations tend to be highly variable as well (Bach et al., 2005). Two factors that have been reported to be correlated are peptide abundance, consistent with the concept that peptide incorporation spares energetic carbon use, and passage rate, which could indicate lower microbial maintenance costs due to less microbial

turnover within the rumen (Oba and Allen, 2003). Also, the source of carbohydrate has been linked to differences in efficiency. For example, high efficiencies have been observed for corn silage versus alfalfa based diets and for those with corn or barley grain (Hristov et al., 2004). It is possible that inclusion of N limitations, fermentative characteristics of the carbohydrate, and passage rates could reduce the variation in microbial efficiency.

In a series of continuous culture fermentation experiments, increasing rumen degradable protein (RDP) up to 19.2% of DM increased bacterial yield and microbial efficiency (Stokes et al., 1991b). However, this level is not practical in feeding dairy cattle, and the effects of increasing RDP with NSC on microbial efficiency is more difficult to observe in vivo (Stokes et al., 1991a). Or if a similar effect is observed, it may be a quadratic effect rather than a simple linear effect, as seen in cows consuming tallgrass-prairie forage and intraruminally infused with casein (Koster et al., 1996). Some of the discrepancies among *in vitro* compared to *in vivo* studies are related to the lack of recycled urea N and/or endogenous protein sources in *in vitro* continuous culture fermentations. This could have been one of the reasons for discrepancy in bacterial N flow with different RDP levels in cattle vs. continuous culture fermenters in a study by Fu et al. (2001).

The Nutrient Requirements of Dairy Cattle publication (NRC, 2001) calculates microbial growth based on total digestible nutrients (TDN); (130 g microbial CP/kg TDN). Since TDN is not homogenous, this approach is not sensitive to differences in the fermentative quality of the energetic substrate. However, the approach does appear to give relatively accurate results. This is because the data from which the empirical equations were based on are diets similar to those fed using the program. In this case, the impact of variability in TDN and in microbial efficiencies with different substrates is minimized. There appears to be a negative correlation of microbial

efficiency and ruminal N balance ($r^2 = 0.41$), and microbial efficiency is 29.7 g N/kg fermentable organic matter (FOM) at a ruminal N balance of zero. However, ruminal N balances within 20% of a zero balance would produce an efficiency of microbial protein synthesis (EMPS) between 25 and 35 g bacterial N/kg FOM, which seems to produce adequate results in this model (Bach et al., 2005). To formulate an RDP requirement, it is assumed that the efficiency of capture of RDP for microbial protein synthesis is 85%. Therefore, the RDP requirement is 1.18*microbial N. If the intake of RDP is less than this, microbial yield is corrected by the amount of RDP (85% of RDP intake). However, application of this is somewhat confounded because the 2001 NRC program assumes all RDP is of dietary origin with no provision for endogenous or recycled N supply. Therefore, microbial efficiencies, and thus microbial growth, could be underestimated at low ruminal N balances.

The CNCPS calculates microbial growth in a much different fashion. It is based on the degradation of specific carbohydrate fractions. Nitrogen availability has the potential to limit growth, and peptides have the ability to improve yield efficiencies on the carbohydrate substrate (Russell et al., 1992). The maximum microbial yield is 50 g microbial cells/100 g degraded CHO, based on the Pirt equation (Pirt, 1965). This is obviously much higher than that proposed by NRC 2001, but it reflects the differences in the calculation of energy availability and utilization by these two systems.

Rumen Degradable Protein

The amount of rumen degradable protein (RDP) and the rate of degradation of the protein in part determine the amount of N available to microbes. Several experiments have used different levels of dietary RDP in an attempt to define the "optimal" proportion. Using data from 38 studies that evaluated milk response to RDP, the NRC 2001 suggested the optimal RDP to be 12.2% of DM. Typically, small increases in

milk yields are seen between large differences in RDP levels, but not between smaller increments of 1 or 2% of the DM (Armentano et al., 1993; Hristov et al., 2004; Kalscheur et al., 2006). The effect of higher RDP would potentially increase microbial protein synthesis, but this small increase in microbial N duodenal flow may be masked by the feed that escapes rumen degradation (RUP), especially if fed at high intake levels. This makes it difficult to determine the an optimal RDP level for cattle, as experimental evidence has produced a variety of results (Broderick, 2003; Kalscheur et al., 2006; Cabrita et al., 2003). In addition, dry matter intake (DMI) may increase as microbes are able to grow and degrade fiber better with more N, confounding results.

In the NRC (2001), the equation DMI = 14.4 + 0.58*RDP was shown to describe the effect of RDP on DMI with a correlation of 0.35. This would indicate that DMI would be 1.7 kg/d less if the diet were 3 percentage units lower than the equation constant, which is a common RDP level. Carbohydrate may also affect the optimal RDP, as more N is able to be utilized with the presence of higher fermentable energy (Stokes et al., 1991b). These interactions can lead to inconclusive results in milk production based of RDP levels, and may explain the difficulties in defining a sufficient amount of RDP. Another confounding factor is the colinearity of decreasing structural carbohydrate and increasing CP content of particular feeds. For example, the NRC (2001) publication discussed the influence of RDP on DMI. However, with forages like alfalfa, as the NDF decreases the CP content usually increases causing potentially confounding relationships between DMI and protein content.

High RDP levels may also contribute to large amounts of excreted N, so one must weigh the potential benefits from feeding a higher RDP level with its corresponding increase in N excretion. Typically, fecal N excretion varies only slightly between diets differing in RDP intake (Figure 7). Increases could be explained by an increase in

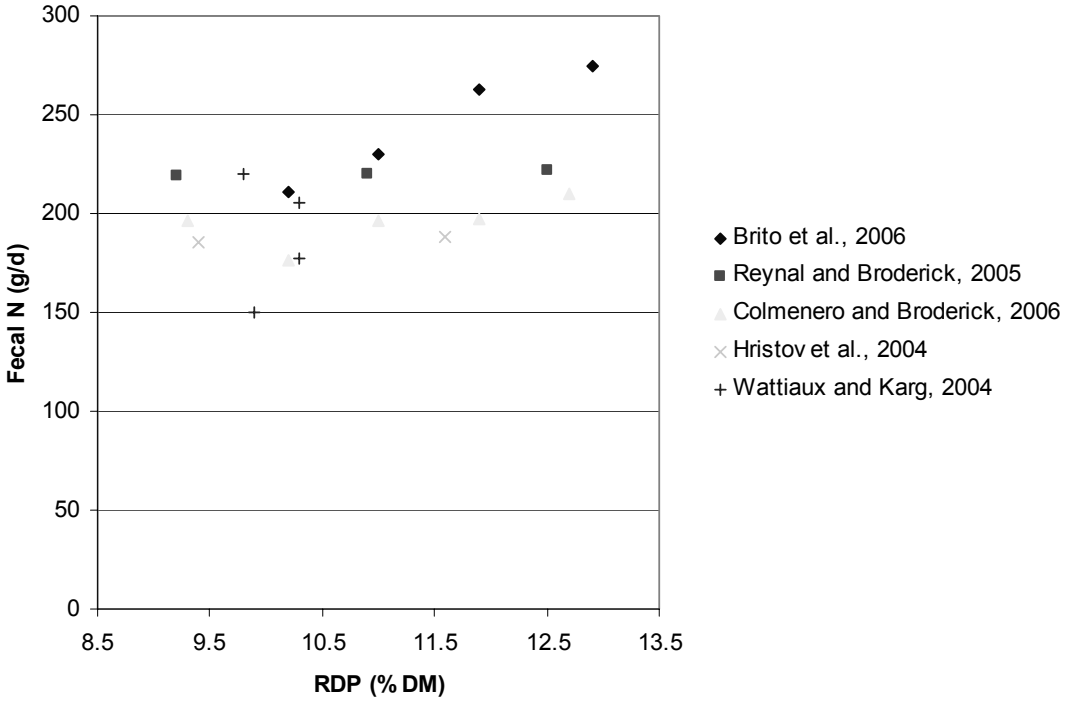

Figure 7. Fecal N output in relation to RDP as % of DM.

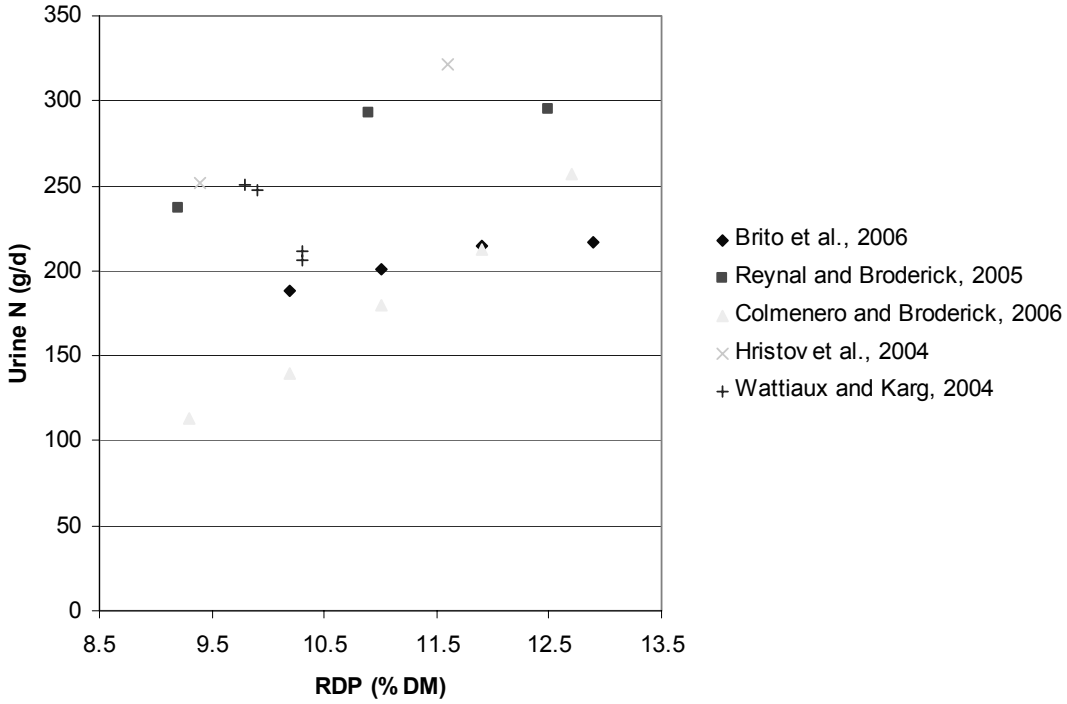

Figure 8. Urine N output in relation to RDP as % of DM.

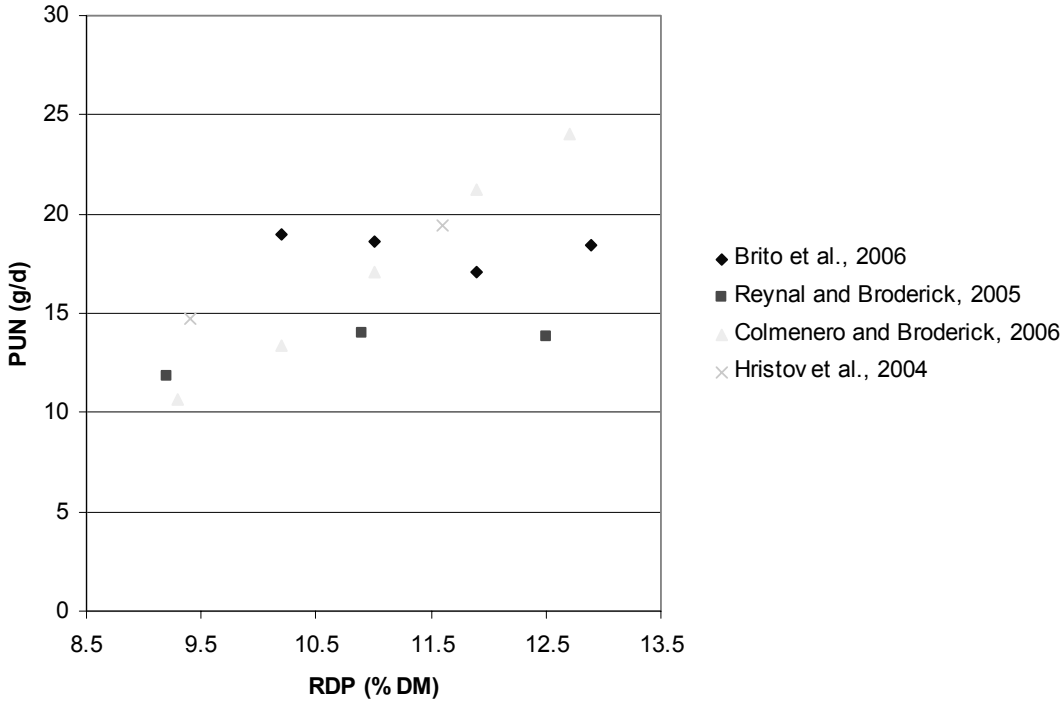

Figure 9. Plasma urea N output in relation to RDP as % of DM.

plasma urea entry into the hindgut, microbial N from hindgut fermentation, and undegraded protein that passes through both the rumen and the hindgut. Urinary N excretion is more responsive than fecal N to N intake, particularly to RDP (Figure 8). This is due to more NH_3 having the potential to be absorbed through the rumen wall, converted to urea, and excreted from the plasma by the kidney. This intermediate step in the excretion process can be observed through plasma urea concentrations (Figure 9). Diets high in CP generally will have an excess rumen N balance, which will be observed as high plasma and urinary urea levels, although the high plasma urea levels are not strictly related to high RDP levels but may be more closely related N in excess of utilization (Elrod et al., 1993).

Bacterial and Protozoal Transactions

A representation of N transactions related to ruminal bacteria is depicted in Figure 10. Bacterial N can be formed from peptides, AA, or NH_3. It can either exit the rumen, providing microbial protein to the intestines, or be retained, which either faces engulfment by protozoa or autolysis. Each of these methods of death eventually lead to reformation of NAN or NH_3, which can then be reincorporated into new bacterial N.

Ruminal protozoa N and its associated pools are shown in Figure 11. Its structure is very similar to bacterial N transactions, except protozoa are assumed to not utilize NH_3 and not be engulfed. Therefore, protozoal N is formed from bacterial N and free peptides and AA. Nitrogen leaves the pool by either exiting the rumen or by cell lysis. This again releases NAN and NH_3, which can be used by both microbial populations for growth or can be utilized for protozoal growth directly. Also, it can reenter into the protozoal population after initial incorporation into bacteria, forming a cycle

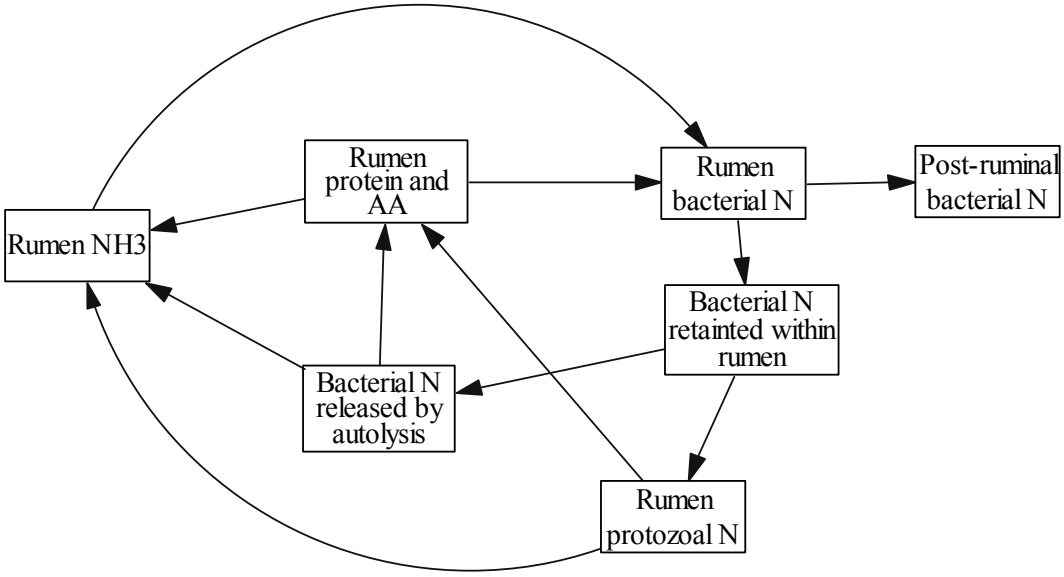

Figure 10. Bacterial N transactions in the rumen.

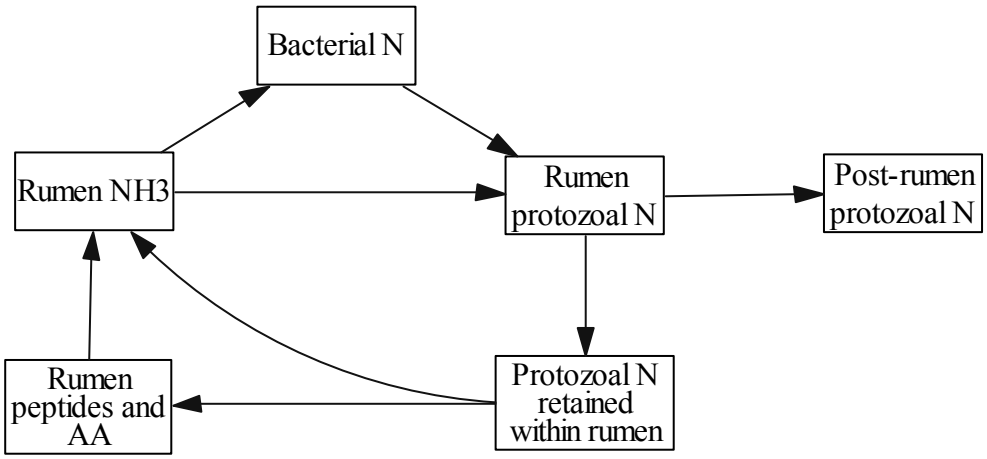

Figure 11. Protozoal N transactions in the rumen.

between these two microbes that may be helpful in sequestering N, especially in its inorganic form.

Due to the difficulty of isolating protozoa and a traditional assumption of their unimportance to the N supply of the animal, little experimental data is available to understand protozoal flows. They can contribute up to 40% of the rumen microbial biomass, so this obviously represents a large proportion of the potential N transactions that occur in the rumen (Russell and Rychlik, 2001). Protozoa tend to bind to feed particles and may be selectively retained in the rumen (Dijkstra, 1994; Shabi et al., 2000). Therefore, this may help explain why their duodenal N flow is typically less than their proportional rumen microbial N mass. Several studies have measured the contribution of protozoal N to the total duodenal flow, and it usually ranges between 10 and 40% of the total protein flow (Jouany, 1996; Shabi et al., 2000; Steinhour et al., 1982).

Protozoa are difficult to separate from the rest of the rumen contents; thus, researchers have searched for techniques that will allow for more accurate and precise measurements of this population. In particular, a method involving straining with cheesecloth, incubating in buffer, and centrifuging the fluid appears to hold promise (Sylvester et al., 2004). When genomic studies were used to examine sample fractions from several isolation techniques, it was found that this method produced cleaner results than methods used previously (Sylvester et al., 2005). The greatest advance was a better separation of bacteria and protozoa, as often protozoal samples normally contain large amounts of bacteria. With this technique, protozoal N was much lower than previously reported, being only 4.8 and 12.7% of the microbial N and 5.9 and 11.9% of the duodenal microbial N flow in low and high forage diets, respectively (Sylvester et al., 2005). It is likely that previous protozoal N quantifications had bacterial contamination, leading researchers to overestimate rumen mass and flow.

Protozoa also play a major role in the digestion of feed particles, particularly starch. In defaunated animals, organic matter (OM) and N digestibility is decreased, causing less to be fermented in the rumen and more to exit it (Koenig et al., 2000). It appears that protozoa allow for a more complete consumption of feed ingredients. However, their impact on microbial growth and supply to the animal is somewhat less well defined. Various defaunation experiments have come to different conclusions of the benefits of these microbes. Most studies report microbial N flows to be increased by the absence of protozoa, and possibly less microbial turnover in the rumen (Koenig et al., 2000). But these estimates may be misleading, since studies that have measured duodenal flow of microbial N include protozoa. In faunated animals, this value could be underestimated due to the purine:N ratio being lower for protozoa than for bacteria, thereby underestimating their contribution. From these studies, it would seem beneficial to eliminate protozoa. However, since the flows of protozoal N out of the rumen and its contribution to N retention and utilization in the rumen environment have not been well defined, it may be too hasty to consider this a positive action.

Microbial Turnover

Microbes either die and lyse on their own impulse or are engulfed by protozoa. This adds complexity into tracing N pathways in the rumen, since this microbial N is then reintroduced to the rumen N pools, which can then follow any outflow path, including resynthesis into microbial protein. Various studies were conducted in the 1970s and 1980s with ^{15}N to quantify these paths, mainly by J. V. Nolan. It was estimated that 30 to >50% of the microbial N was recycled in sheep (Leng and Nolan, 1984). However this was recycling only through the NH_3 pool, so it may underestimate total recycling, as NAN may also be recycled without passing through the NH_3 pool (Firkins et al., 1992). A later study found recycling of microbial NAN to

be 78 to 91% in nonlactating cows and heifers (Firkins et al., 1992), which is too high of a turnover rate due to measured microbial growth and passage rates (Wells and Russell, 1996). It is apparent that this cycle can play a major role in the sequestering and providing of N in the rumen, and it should be accounted for when considering N use and availability in microbial populations.

Recycled Nitrogen

After absorption in the gastrointestinal tract (GIT), almost all NH_3 is converted to urea in the liver, which can then either be excreted by the kidneys or reenter the GIT. This can increase the supply of digestible N by 50 to 60% in dairy cows (Lapierre and Lobley, 2001). A review of literature showed that urea N synthesis ranged from 43 to 123% of the digested N in lactating dairy cows. Of this plasma urea, approximately two-thirds of it reentered the GIT, where it was rapidly hydrolyzed to form NH_3 (Lapierre and Lobley, 2001).

The stable isotope of N, ^{15}N, has been used to quantify N recycling from the plasma to the GIT in ruminants by infusing doubly labeled urea into the plasma until plateau is achieved (Lobley et al., 2000). Using this technique, in lactating dairy cows fed 60% forage at 14.5% CP and producing 94-100 g milk N/d, an average of 128 g N/d reentered the GIT (Ouellet et al., 2004). In a similar study with cows fed corn grain and slightly higher CP levels (15.7% CP with or without 15 g urea/kg DMI), the animals recycled 381 g N/d back into the GIT (Lapierre et al., 2004). Using a different technique based on a bolus dose of ^{15}N, 118 and 177 g N/d of the NH_3 pool were recycled in cows consuming 23.7 kg/d of a diet based on alfalfa hay, tricale silage, and corn grain (Hristov et al., 2004). Even though these results are varied, they demonstrate that a large proportion of the intake N is able to be reused by microbes after reentry of urea into the GIT.

Differences in the partitioning of recycled N between the rumen and hindgut and the total amount recycled may be influenced by fermentable energy sources. Increases in energy fermentation result in corresponding increases in the influx of urea N across the GIT walls (Kennedy, 1980). Similarly, higher fermentative capacity in the rumen shifts urea N partitioning between the rumen and the hindgut (Kennedy and Milligan, 1980). However, further research needs to be done to fully elucidate this relationship between urea reentry and energetic status. Since this urea N then enters the rumen NH_3 pool, it can be used in the same manner. In a study using lactating dairy cattle, 45 % of the urea N synthesized was used for anabolic purposes (Ouellet et al., 2004). Obviously, recycled N can play a major role in retaining N within the animal and providing microbes with a larger net supply of NH_3.

Endogenous Nitrogen

Amino acids passing to the duodenum originate from undegraded feed, microbial protein, and endogenous secretions. These are AA from the animal, such as GIT epithelial cells, saliva mucoproteins, and enzymatic secretions. As these AA combine with those from several other sources, it is difficult to quantify them. Some attempts have been made by supplying protein free diets, but these numbers are greatly biased by GIT tissue reabsorption, lower maintenance costs, and abnormal microbial fermentation (Whitelaw et al., 1986). More recently, (Ouellet et al., 2002) infused Holstein cows (607 kg BW, 203 DIM, 17.3 kg/d milk yield) restricted at 90% of DMI with ^{15}N leucine for nine days to measure endogenous flows. Endogenous N into the forestomachs and the intestines averaged 78 and 23 g N/d, respectively. Urea contributed 46 g N/d into the forestomach. Bacteria utilized 30.5 and 37.5 g N/d from the endogenous and urea sources, respectively. This N was then passed into the duodenum either in free form (32.5 g N/d) or in bacteria (30.5 g N/d), which

contributed 15% of the duodenal N flow. Because these cows had a very low DMI and production status, it would be valuable to repeat similar experiments with higher yielding cows.

Due to a relationship between indigestible OM intake, the NRC (2001) determines the grams (g) of endogenous N produced per day as 1.9*DMI. This equation predicted 27 g N/d in the previously mentioned study, which underestimated the measured value by 74 g N/d, or 274%. This endogenous protein secreted into the rumen can then be used for microbial protein synthesis or exit the rumen to supply MP to the animal and represents another form of recycling not generally accounted for in routine ration balancing software.

Monensin

Monensin is an ionophore that inserts into biological membranes and transports metal ions, specifically causing an efflux of potassium ions and an influx of hydrogen and sodium ions (Figure 12). This upsets the ionic equilibrium, and the cell rapidly expends energy to reestablish it, killing itself or retarding it growth in the process (Russell, 2002). There are several effects this ionophore is purported to have when fed to ruminants, mainly relating to effects regarding changes to the bacterial population profile, which consequently affect the animal.

Because most of the bacterial species identified thus far that can convert trans-18:1 to 18:0 fatty acids are gram positive, it is likely that this leads to a build up of these biohydrogenation intermediates in the rumen (Firkins et al., 2006; Lock et al., 2006). In continuous culture fermenters, an addition of monensin decreased the rate of production of stearic acid (from 7.5 mg/L in controls to 1.4 mg/L with monensin) and increased concentrations of its polyunsaturated fatty acid (PUFA) precursors (Fellner et al., 1997). Thus, this may explain the reduction in milk fat commonly observed

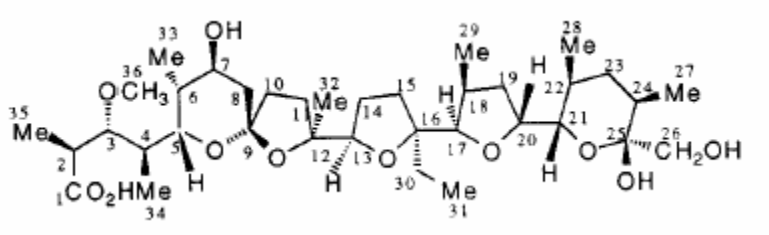

Figure 12. The chemical structure of monensin. Adapted from (Riddell, 2002).

with monensin feeding. However, there must be a supply of PUFAs in order for milk fat depression (MFD) to occur. Since corn silage is often high in linoleic acid, this ingredient can supply enough substrate for MFD.

Ruminal pH is typically lower in ruminants fed monensin, possibly due to its effect of reducing lactic acid-fermenting bacteria while enhancing lactic acid utilizers (Duffield and Bagg, 2000). It may also enhance the probablility of MFD, as these bacterial population shifts may be favorable for altering biohydrogenation pathways, but this is not always an indicator that MFD will occur.

Due to this altered fermentation status caused by monensin, the substrate profile presented to the animal will be different, as well as the nature of the exchanges between the rumen and the rest of the animal. The volatile fatty acid (VFA) profile typically changes, with higher proportions of propionic acid and lower proportions of butyric and acetic acid, which may enhance gluoconeogenesis (Duffield and Bagg, 2000).

Researchers have identified Gram-positive, monensin-sensitive bacteria with very high specific activity of NH_3 production (Chen and Russell, 1989). Monensin reduces numbers of these bacteria, such as observed by (Yang and Russell, 1993), who noticed a 10-fold decrease in the presence of monensin fed to cattle at 350 mg/d. In a later study, of the 19 bacterial isolates capable of growing on trypticase and demonstrating high NH_3 production capacity (1.4% of the total bacterial population), 93% were eliminated by 5 uM monensin. In addition to, and most likely a result of, the reduction in bacterial numbers, monensin reduces NH_3 concentrations (Yang and Russell, 1993). This NH_3 reduction is proportional to the amount of substrates, as the NH_3 concentrations were 28% lower with proteins, peptides, and AA added at 2 mg/ml and 48% added at 20 mg/ml (Eschenlauer et al., 2002).

This reduction in peptide and AA use potentially stimulated the carbohydrate-fermenting bacteria due to the protein "sparing effect", allowing for greater bacterial N growth (Chen and Russell, 1991; Eschenlauer et al., 2002). Therefore, it is possible that monensin can increase N flow out of the rumen due to an increase in undegraded feed protein, protein and AA from microbial lysis and endogenous secretions that were not deaminated, and from a larger amounts of bacterial N. Monensin has been shown to increase feed efficiency and average daily gain in animals fed diets supplemented with potentially degradable protein but not with urea (Lana et al., 1997). This N can be degraded post-ruminally and may cause the higher plasma urea concentrations often observed in monensin fed animals (Duffield et al., 2003; Duffield and Bagg, 2000; Stephenson et al., 1997). So, the total amount of degraded N, rather than the ruminally degraded N, may be a better predictor of the animal's N status, and therefore, its N excretion profile (Elrod et al., 1993).

Putting It All Together: Perturbing an Example

The main issue this thesis calls into question is, "How much N is available in the rumen?" In essence this question is composed of two major points: the amount of N entering and becoming available in the rumen and the outflow of N from the rumen pool by microbial utilization, passage, and absorption. By definition, the amount of N required for microbial growth is based on the amount of fermentable energy. Therefore, one can assume there exists a certain amount of energetic material to examine what occurs by varying the amount of N presented with it. If the model is not accounting for all available N, how deficient in rumen N, as currently calculated, could rations be formulated for to actually achieve a zero rumen N balance?

Reexamining the N flow diagrams, there are several N inflows to the rumen: bacteria, protozoa, feed, plasma, and endogenous N sources (Figure 13). The amount

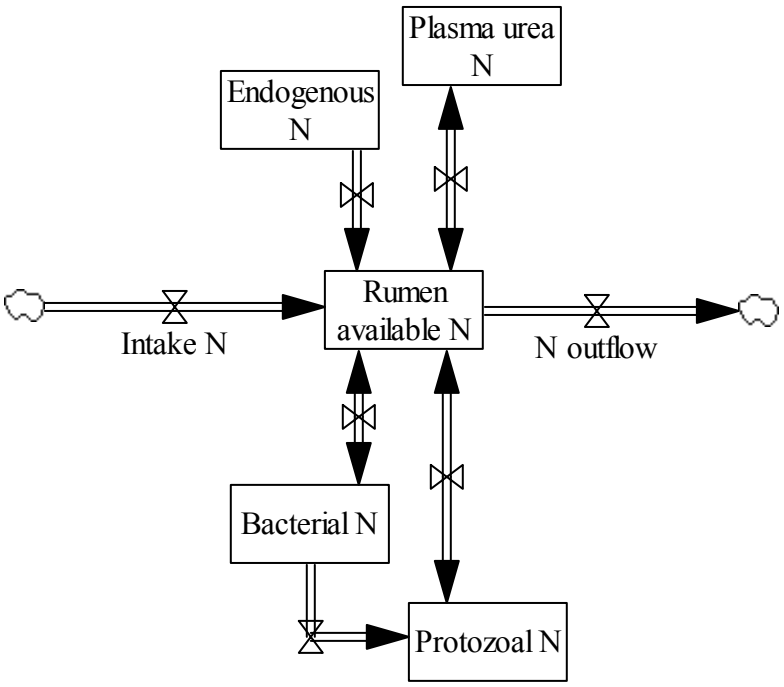

Figure 13. Ruminal N transactions to consider when determining the amount of ruminally available N.

of feed N is dependent on its chemical composition and degradation rates. Each model has its own approach to calculate the appearance of N in its various forms depending on these values, but overall, they arrive at similar values as the mechanics behind the numeric inputs are the same. Therefore, this source will not be focused on in this thesis.

Bacterial and protozoal turnover has not been very well quantified, but it may be 30 to 50% of the microbial N. In a cow with a rumen microbial N pool of 180 g, (Sylvester et al., 2005) this means 54 to 90 g of that N could be recycled. Since the grams of microbial N flow out of the rumen are generally twice that much, due to rumen material turnover, even higher amounts of microbial N are recycled each day. Calculating the impact of microbial turnover is difficult, since most models are built on empirical data that already has some accounting for this factor inherently in it. For example, the amount of microbial N able to be produced from a certain amount of TDN, carbohydrate (CHO), etc. has been assigned from experimental observation that includes the normal process of microbial turnover. The CNCPS reduces the efficiency of CHO use for microbial N by 20% (from 50 to 40 g cells/100 g CHO degraded) due to protozoal predation. It may be possible, and more correct, to assign values without including turnover impacts, but this would require much more quantitative work in this specific area. At present, one must accept potentially reasonable values while considering that more N may be available, specifically in the form of peptides and AA.

Endogenous N is currently not accounted for in CNCPS (Fox et al., 2004), but it is by NRC (2001) as stated previously. The amount of endogenous N secretions into the rumen in high producing dairy cows is currently unknown, but it is most likely much more than that currently predicted by the NRC model. For instance, an additional 50 g

endogenous N/d, if used with an efficiency of 85% and assuming 130 g microbial N/kg TDN, could allow for usage of an additional 0.33 kg TDN for microbial growth, or 42.5 g microbial N. This endogenous supply would be added into the ruminal peptide balance and could also lead to growth stimulation by peptides.

Recycled N through plasma urea is able to increase the amount of N available to the microbes by presenting it to them for use multiple times. Based on the NRC (1985) equation, $121.7 - 12.1*CP + 0.3235*CP^2$ = g of recycled N as % of N intake, a 15% CP diet would recycle only 14% of the N intake. However, studies have indicated this value to be closer to 39 to 76% of the N intake (Lapierre et al., 2004; Lapierre and Lobley, 2001; Ouellet et al., 2004). Clearly, this has a tremendous impact on the ruminal N balance, and it could be used for microbial protein synthesis if energy conditions were favorable.

With all these considerations in mind, it might be possible to feed cattle much below the currently recommended ruminal N balances in order to improve overall N efficiency and reduce the environmental impact of cattle. More research needs to be conducted to elucidate the numbers, but in the present one can experiment with knowledge of these additional, unaccounted for, factors in mind.

REFERENCES

Armentano, L. E., S. J. Bertics, and J. Riesterer. 1993. Lack of response to addition of degradable protein to a low protein diet fed to midlactation dairy cows. J. Dairy Sci. 76:3755-3762.

Atasoglu, C., C. J. Newbold, and R. J. Wallace. 2001. Incorporation of ^{15}N ammonia by the cellulolytic ruminal bacteria Fibrobacter succinogenes BL2, Ruminococcus albus SY3, and Ruminococcus flavefaciens 17. Appl. Environ. Microbiol. 67:2819-2822.

Bach, A., S. Calsamiglia, and M. D. Stern. 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88(Suppl 1):E9-21.

Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370-1381.

Cabrita, A. R. J., A. J. M. Fonseca, R. J. Dewhurts, C. V. P. Sampaio, M. F. S. Miranda, G. N. S. Sousa, I. M. F. Miranda, and E. Gomes. 2003. Nitrogen supplementation of corn silages. 1. Effects on feed intake and milk production of dairy cows. J.Dairy Sci. 86:4008-4019.

Chen, G. and J. B. Russell. 1989. More monensin-sensitive, ammonia-producing bacteria from the rumen. Appl. Environ. Microbiol. 55:1052-1057.

Chen, G., C. J. Sniffen, and J. B. Russell. 1987. Concentration and estimated flow of peptides from the rumen of dairy cattle: effects of protein quantity, protein solubility, and feeding frequency. J. Dairy Sci. 70:983-992.

Chen, G. J. and J. B. Russell. 1991. Effect of monensin and a protonophore on protein degradation, peptide accumulation, and deamination by mixed ruminal microorganisms in vitro. J. Anim Sci. 69:2196-2203.

Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304-2323.

Cocimano, M. R. and R. A. Leng. 1967. Metabolism of urea in sheep. Br. J. Nutr. 21:353-371.

Cruz Soto, R., S. A. Muhammed, C. J. Newbold, C. S. Stewart, and R. J. Wallace. 1994. Influence of peptides, amino acids and urea on microbial activity in the rumen of sheep receiving grass hay and on the growth of rumen bacteria in vitro. Anim.Feed Sci.Tech. 49: 151-161.

Di, H. J. and K. C. Cameron. 2002. Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. Nutrient Cycling in Agroecosystems 64:237-256.

Diaz, M. C., M. E. Van Amburgh, J. M. Smith, J. M. Kelsey, and E. L. Hutten. 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105-kilogram body weight. J Dairy Sci 84:830-842.

Dijkstra, J. 1994. Simulation of the dynamics of protozoa in the rumen. Br. J. Nutr. 72:679-699.

Duffield, T. F. and R. N. Bagg. 2000. Use of ionophores in lactating dairy cattle: a review. Can. Vet. J. 41:388-394.

Duffield, T. F., S. LeBlanc, R. Bagg, K. Leslie, J. Ten Hag, and P. Dick. 2003. Effect of a monensin controlled release capsule on metabolic parameters in transition dairy cows. J. Dairy Sci. 86:1171-1176.

Elrod, C. C., M. Van Amburgh, and W. R. Butler. 1993. Alterations of pH in response to increased dietary protein in cattle are unique to the uterus. J Anim Sci 71:702-706.

Eschenlauer, S. C., N. McKain, N. D. Walker, N. R. McEwan, C. J. Newbold, and R. J. Wallace. 2002. Ammonia production by ruminal microorganisms and enumeration, isolation, and characterization of bacteria capable of growth on peptides and amino acids from the sheep rumen. Appl. Environ. Microbiol. 68:4925-4931.

Fellner, V., F. D. Sauer, and J. K. Kramer. 1997. Effect of nigericin, monensin, and tetronasin on biohydrogenation in continuous flow-through ruminal fermenters. J. Dairy Sci. 80:921-928.

Firkins, J. L., L. L. Berger, N. R. Merchen, G. C. Fahey, Jr., and R. L. Mulvaney. 1987. Ruminal nitrogen metabolism in steers as affected by feed intake and dietary urea concentration. J. Dairy Sci. 70:2302-2311.

Firkins, J. L., A. N. Hristov, M. B. Hall, G. A. Varga, and N. R. St Pierre. 2006. Integration of ruminal metabolism in dairy cattle. J. Dairy Sci. 89(Suppl 1):E31-E51.

Firkins, J. L., W. P. Weiss, and E. J. Piwonka. 1992. Quantification of intraruminal recycling of microbial nitrogen using nitrogen-15. J. Anim Sci. 70:3223-3233.

Fox, D. G., L. O. Tedeschi, T. P. Tylutki, J. B. Russell, M. E. Van Amburgh, L. Chase, A. N. Pell, and T. R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim.Feed Sci.Tech. 112:29-78.

Fu, C. J., E. E. Felton, J. W. Lehmkuhler, and M. S. Kerley. 2001. Ruminal peptide concentration required to optimize microbial growth and efficiency. J. Anim Sci. 79:1305-1312.

Groff, E. B. and Z. Wu. 2005. Milk production and nitrogen excretion of dairy cows fed different amounts of protein and varying proportions of alfalfa and corn silage. J Dairy Sci 88:3619-3632.

Hanigan, M. D., J. P. Cant, D. C. Weakley, and J. L. Beckett. 1998. An evaluation of postabsorptive protein and amino acid metabolism in the lactating dairy cow. J Dairy Sci 81:3385-3401.

Hoover, W. H. and S. R. Stokes. 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. J. Dairy Sci. 74:3630-3644.

Hristov, A. N., R. P. Etter, J. K. Ropp, and K. L. Grandeen. 2004. Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. J. Anim Sci. 82:3219-3229.

Ipharraguerre, I. R. and J. H. Clark. 2005. Varying protein and starch in the diet of dairy cows. II. Effects on performance and nitrogen utilization for milk production. J. Dairy Sci. 88:2556-2570.

Jonker, J. S., R. A. Kohn, and J. High. 2002. Dairy herd management practices that impact nitrogen utilization efficiency. J. Dairy Sci. 85:1218-1226.

Jouany, J. P. 1996. Effect of rumen protozoa on nitrogen utilization by ruminants. J. Nutr. 126:1335S-1346S.

Kalscheur, K. F., R. L. Baldwin, B. P. Glenn, and R. A. Kohn. 2006. Milk production of dairy cows fed differing concentrations of rumen-degraded protein. J. Dairy Sci. 89:249-259.

Kennedy, P. M. 1980. The effects of dietary sucrose and the concentration of plasma urea and rumen ammonia on the degradation of urea in the gastrointestinal tract of cattle. Br. J. Nutr. 43:125-140.

Kennedy, P. M. and L. P. Milligan. 1980. The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: a review. Canadian J.Anim.Sci. 60:205-221.

Koenig, K. M., C. J. Newbold, F. M. McIntosh, and L. M. Rode. 2000. Effects of protozoa on bacterial nitrogen recycling in the rumen. J. Anim Sci. 78:2431-2445.

Koster, H. H., R. C. Cochran, E. C. Titgemeyer, E. S. Vanzant, I. Abdelgadir, and G. St Jean. 1996. Effect of increasing degradable intake protein on intake and

digestion of low-quality, tallgrass-prairie forage by beef cows. J. Anim Sci. 74:2473-2481.

Lana, R. P., D. G. Fox, J. B. Russell, and T. C. Perry. 1997. Influence of monensin on Holstein steers fed high-concentrate diets containing soybean meal or urea. J. Anim Sci. 75:2571-2579.

Lapierre, H., R. Berthiaume, G. Raggio, M. C. Thivierge, L. Doepel, D. Pacheco, P. Dubreuil, and G. E. Lobley. 2005. The route of absorbed nitrogen into milk protein. Animal Science 80:11-22.

Lapierre, H. and G. E. Lobley. 2001. Nitrogen recycling in the ruminant: a review. J.Dairy Sci. 84(E. Suppl.): E223-E236.

Lapierre, H., D. R. Ouellet, R. Berthiaume, C. Girard, P. Dubreuil, M. Babkine, and G. E. Lobley. 2004. Effect of urea supplementation on urea kinetics and splanchnic flux of amino acids in dairy cows. J.Anim.Feed Sci. 13(Suppl. 1):319-322.

Leng, R. A. and J. V. Nolan. 1984. Nitrogen metabolism in the rumen. J. Dairy Sci. 67:1072-1089.

Lobley, G. E., D. M. Bremner, and G. Zuur. 2000. Effects of diet quality on urea fates in sheep as assessed by refined, non-invasive ($^{15}N^{15}N$)urea kinetics. Br. J. Nutr. 84:459-468.

Lock, A. L., T. R. Overton, K. J. Harvatine, J. Giesy, and D. E. Bauman. 2006. Milk fat depression: Impact of dietary components and their interaction during rumen fermentation. Pages 75-86 in Cornell Nutr. Conf. Proc., Syracuse, NY.

Macrae, J. C., A. Walker, D. Brown, and G. E. Lobley. 1993. Accretion of total protein and individual amino acids by organs and tissues of growing lambs and the ability of nitrogen balance techniques to quantitate protein retention. Anim.Prod. 57:237-245.

National Academies Press. 2003. Air emissions from animal feeding operations: Current knowledge, future needs. Washington, DC.

National Research Council. 1985. Ruminant Nitrogen Usage. Washington, DC, National Academy Press.

National Research Council. 2001. Nutritional Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Noftsger, S. and N. R. St Pierre. 2003. Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. J. Dairy Sci. 86:958-969.

Nolan, J. V. 2005. Quantitative models of nitrogen metabolism in sheep. Pages 416-431 in Digestion and Metabolism in the Ruminant. University of New England. Armidale, NSW, Australia.

Oba, M. and M. S. Allen. 2003. Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. J. Dairy Sci. 86:195-207.

Oldham, J. D. 1984. Protein-energy interrelationships in dairy cows. J. Dairy Sci. 67:1090-1114.

Ouellet, D. R., R. Berthiaume, G. E. Lobley, R. Martineau, and H. Lapierre. 2004. Effects of sun-curing, formic acid-treatment or microbial inoculation of timothy on urea metabolism in lactating dairy cows. J.Anim.Feed Sci. 13(Suppl. 1): 323-326.

Ouellet, D. R., M. Demers, G. Zuur, G. E. Lobley, J. R. Seoane, J. V. Nolan, and H. Lapierre. 2002. Effect of dietary fiber on endogenous nitrogen flows in lactating dairy cows. J. Dairy Sci. 85:3013-3025.

Pirt, S. J. 1965. The maintenance energy of bacteria in growing cultures. Proc.R.Soc.Lond.Ser.B Biol.Sci. 163-224.

Riddell, F. G. 2002. Structure, conformation, and mechanism in the membrane transport of alkali metal ions by ionophoric antibiotics. Chirality 14:121-125.

Ruiz, R., L. O. Tedeschl, J. C. Marini, D. G. Fox, A. N. Pell, G. Jarvis, and J. B. Russell. 2002. The effect of a ruminal nitrogen (N) deficiency in dairy cows: evaluation of the cornell net carbohydrate and protein system ruminal N deficiency adjustment. J. Dairy Sci. 85:2986-2999.

Russell, J. B. 1983. Fermentation of Peptides by Bacteroides ruminicola B(1)4. Appl. Environ. Microbiol. 45:1566-1574.

Russell, J. B. 2002. Rumen Microbiology and Its Role in Ruminant Nutrition. James B. Russell, Ithaca, NY.

Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim Sci. 70:3551-3561.

Russell, J. B. and J. L. Rychlik. 2001. Factors that alter rumen microbial ecology. Science 292:1119-1122.

Russi, J. P., R. J. Wallace, and C. J. Newbold. 2002. Influence of the pattern of peptide supply on microbial activity in the rumen simulating fermenter (RUSITEC). Br. J. Nutr. 88:73-80.

Salter, D. N., K. Daneshvar, and R. H. Smith. 1979. The origin of nitrogen incorporated into compounds in the rumen bacteria of steers given protein- and urea-containing diets. Br. J. Nutr. 41:197-209.

Satter, L. D. and L. L. Slyter. 1974. Effect of ammonia concentration of rumen microbial protein production in vitro. Br. J. Nutr. 32:199-208.

Shabi, Z., H. Tagari, M. R. Murphy, I. Bruckental, S. J. Mabjeesh, S. Zamwel, K. Celik, and A. Arieli. 2000. Partitioning of amino acids flowing to the abomasum into feed, bacterial, protozoal, and endogenous fractions. J. Dairy Sci. 83:2326-2334.

Steinhour, W. D., M. R. Stokes, J. H. Clark, J. A. Rogers, C. L. Davis, and D. R. Nelson. 1982. Estimation of the proportion of non-ammonia-nitrogen reaching the lower gut of the ruminant derived from bacterial and protozoal nitrogen. Br. J. Nutr. 48:417-431.

Stephenson, K. A., I. J. Lean, M. L. Hyde, M. A. Curtis, J. K. Garvin, and L. B. Lowe. 1997. Effects of monensin on the metabolism of periparturient dairy cows. J. Dairy Sci. 80:830-837.

Stokes, S. R., W. H. Hoover, T. K. Miller, and R. Blauweikel. 1991a. Ruminal digestion and microbial utilization of diets varying in type of carbohydrate and protein. J. Dairy Sci. 74:871-881.

Stokes, S. R., W. H. Hoover, T. K. Miller, and R. P. Manski. 1991b. Impact of carbohydrate and protein levels on bacterial metabolism in continuous culture. J. Dairy Sci. 74:860-870.

St-Pierre, N. R. and C. S. Thraen. Animal grouping strategies, sources of variation, and economic factors affecting nutrient balance on dairy farms. J. Dairy Sci. 82(Suppl. 2):72-83.

Sylvester, J. T., S. K. Karnati, Z. Yu, M. Morrison, and J. L. Firkins. 2004. Development of an assay to quantify rumen ciliate protozoal biomass in cows using real-time PCR. J. Nutr. 134:3378-3384.

Sylvester, J. T., S. K. Karnati, Z. Yu, C. J. Newbold, and J. L. Firkins. 2005. Evaluation of a real-time PCR assay quantifying the ruminal pool size and duodenal flow of protozoal nitrogen. J. Dairy Sci. 88:2083-2095.

Valkeners, D., A. Thewis, S. Amant, and Y. Beckers. 2006. Effect of various levels of imbalance between energy and nitrogen release in the rumen on microbial protein synthesis and nitrogen metabolism in growing double-muscled Belgian Blue bulls fed a corn silage-based diet. J. Anim Sci. 84:877-885.

Valkeners, D., A. Thewis, F. Piron, and Y. Beckers. 2004. Effect of imbalance between energy and nitrogen supplies on microbial protein synthesis and nitrogen metabolism in growing double-muscled Belgian Blue bulls. J. Anim Sci. 82:1818-1825.

VandeHaar, M. J. and N. St Pierre. 2006. Major advances in nutrition: relevance to the sustainability of the dairy industry. J. Dairy Sci. 89:1280-1291.

Wallace, R. J., C. Atasoglu, and C. J. Newbold. 1999. Role of peptides in rumen microbial metabolism. AJAS 12(1), 139-147.

Whitelaw, F. G., J. S. Milne, and X. B. Chen. 1991. The effect of a rumen microbial fermentation on urea and nitrogen metabolism of sheep nourished by intragastric infusion. Exp. Physiol 76:91-101.

Yang, C. M. and J. B. Russell. 1993. Effect of monensin on the specific activity of ammonia production by ruminal bacteria and disappearance of amino nitrogen from the rumen. Appl. Environ. Microbiol. 59:3250-3254.

CHAPTER TWO: EFFECT OF FEEDING CORN SILAGE BASED DIETS PREDICTED TO BE DEFICIENT IN EITHER RUMINAL NITROGEN OR METABOLIZABLE PROTEIN ON NITROGEN UTILIZATION AND EFFICIENCY OF USE IN LACTATING COWS

ABSTRACT

The objective of this experiment was to evaluate nitrogen (N) utilization in high-producing lactating dairy cattle under conditions of a predicted (1) positive rumen N and metabolizable protein (MP) balances (Diet P) (2) negative MP balance and positive N balance (Diet N), or (3) negative rumen N balance but positive MP balance (Diet T) as predicted by CPM Dairy version 3. Eighty-eight multiparous lactating Holstein cows (83 ± 20 DIM), were blocked by average daily milk yield to 50 DIM and parity and assigned to three diets differing in N content or predicted rumen degradability of the feed N. The diets were formulated with CPM Dairy V3 using library values for all feeds except corn silage where actual chemical, digestibility, and degradation rate values were determined and used. The diets consisted of approximately 50% corn silage, 2% wheat straw and 48% of a diet specific ingredient mix and were formulated for 22.2 kg DMI. Actual diet crude protein (CP) levels were 16.7, 14.2 and 14.3% for Diets P, N and T, respectively. The predicted CPM Dairy rumen N balance at the formulated DMI was 29 and 27 g for Diets P and N and negative 39 g/d for Diet T, whereas the predicted MP balance was 263, negative 145 g/d and 91 for Diets P, N and T, respectively. Monensin was included in the diets at a formulated intake of approximately 300 mg per cow per d and somatotropin was administered per label. A transition period of 7 d preceded the treatment period of 100 d. Actual DMI for cattle on these treatments were 25.7, 25.5 and 24.2 kg/d for Diets

P, N and T, respectively and were significantly lower for Diet T. Actual milk yield was 45.0, 42.6 and 43.3 kg/d and 3.5% FCM was 38.1, 36.5, and 36.4 kg/d, for cows fed Diets P, N and T, respectively and was significantly lower for cows fed Diets N and T. Milk protein percent was not affected by diet; however, milk protein yield (kg protein/d) was significantly greater for cows fed Diet P due to the difference in milk yield. There was a significant linear decrease in plasma urea N values, 11.31, 8.40 and 7.13 mg/dl for cows fed diets P, N and T, respectively and these values paralleled the rumen ammonia levels of 8.32, 6.58 and 5.84 mg/dl. Milk fat depression (MFD) was observed in all cows and was not affected by treatment, and the average milk fat levels were 2.67, 2.68 and 2.54% for diets P, N and T, respectively. To determine if monensin was partially responsible for the MFD, monensin was removed from the diets of approximately half of the cows on treatment, once they had finished the experimental period. Removal of monensin resulted in a 30% increase in milk fat percent, and milk yield and protein was not affected. Cows consuming Diet P produced approximately $11 and $43 more net income over feed cost than those consuming Diet N and T, respectively. Calculated milk N:intake N ratios for the three treatments were 0.31, 0.33 and 0.36 for Diets P, N and T respectively. Evaluation of predicted MP supply in CPM Dairy and the Dairy NRC (2001) compared to the actual milk yield suggests more productive N is available than currently predicted by either system.

INTRODUCTION

Ruminants contribute significant amounts of ammonia and nitrous oxide emissions to the atmosphere and surface water (National Academies Press, 2003). These compounds aid in the formation of ozone and particulate matter in the air and in

nitrogen compounds within water supplies. It is imperative that strategies be developed to minimize nitrogen (N) excretion into the environment. The most logical approach to reduce N excretion and improve N efficiency is through improved milk protein output (Jonker et al., 2002), and more accurate nutrient and ration balancing (Borsting et al., 2003).

According to the NRC (National Research Council, 2001), as the amount of crude protein (CP) in the diet of dairy cattle increases, the amount of milk produced increases until it reaches maximal milk yield at approximately 23% CP. Further increases in the amount of CP per diet DM results in decreases in milk yield. However, the amount of excess N (intake N minus milk N, in the case strictly focusing on N use for milk production) increases with corresponding CP increases. Traditionally it has been economically profitable to feed at high CP concentrations in order to achieve the highest possible milk production as the revenues from milk production outweighed the cost of protein feeds to achieve it. St-Pierre and Thraen (1999) averaged the prices of 23 different feed ingredients over 15 years then used the relationships between nutrient intake and milk production by the NRC (National Research Council, 1989) and a variety of statistical procedures to account for variation in BW, maximum production potential, efficiency of nutrient use by the animal, and DMI. Milk production was maximized at 18.6% CP, but N efficiency was maximized at 14.9% CP and income over feed costs was maximized at 18.0% CP. The authors stated that transitioning the feeding strategy of dairy cattle from maximal income over feed costs to maximum N efficiency (milk N:excreted N ratio) would reduce national N excretion by 142,000 tons, but would cost $1.35 billion per year. However, the relationship between N intake and milk yield used in this analysis was based on CP intake rather than RDP and RUP. If the latter were used, it may be possible to refine

N intake in such a way to reduce it without any or less detrimental effects on milk production.

The ratio of N output in milk to N input from feed, which is an estimation of gross N efficiency, is typically 0.20 to 0.35 (Jonker et al., 2002; Ipharraguerre and Clark, 2005; Groff and Wu, 2005; Broderick, 2003). Efficiencies of 0.37 have been reported, but this level is rare (Noftsger and St Pierre, 2003). In a review of literature data it was reported across multiple studies on lactating dairy cows, that on average, 0.35 of the N ingested is excreted in feces, 0.34 in urine, and 0.31 in milk (Lapierre and Lobley, 2001). The waste N, ~70% of intake N, is then distributed into the environment, which can lead to environmental and human health problems, such as acid rain, nitrate contamination in groundwater, and increased particulate matter in the air (NAP, 2003). Thus, for more immediate application, focusing on reducing urinary urea N excretion instead of increased conversion efficiency of feed N to milk N might have greater environmental impact for dairy producers.

Metabolizable protein evaluation and balancing systems, if properly understood, should hold the promise to improve N efficiency. Several studies and a review have demonstrated that the NRC (2001) may be oversensitive to total protein and rumen degradable protein (RDP) supply (Firkins et al., 2006). Recent work has suggested that below approximately 16% CP there is a reduction in milk yield in early to mid-lactation cows (Broderick, 2003; Kalscheur et al., 2006) and that this can be affected by source of forage (Groff and Wu, 2005; Wattiaux and Karg, 2004a). Milk yield and N efficiency data are equivocal concerning the effect of RDP and rumen undegradable protein (RUP) levels (Davidson et al., 2003; Kalscheur et al., 2006; Reynal and Broderick, 2003) suggesting that source of forage (alfalfa versus corn silage) and overall level of N intake obscure some of the potential effects. Under feeding RDP

can reduce microbial yield, fiber digestibility and energy transformations (Clark et al., 1992; Stokes et al., 1991b).

The Dairy NRC (2001) suggests greater microbial yield with increasing RDP supply and maximum microbial yield responses at RDP levels greater than 12% of CP. However, this has been called into question based on a reevaluation of the data used to establish that value (Firkins et al., 2006). For example, Weiss and Wyatt (2006), observed no effect of protein supply on milk or milk component yield for cows fed at 85% and 115% of predicted MP supply. Further, a 13 kg/d difference in predicted MP allowable milk yield due to MP (89 to 114% of required) and RDP (68 to 111% of required) supply resulted in only a 2 kg/d difference in actual milk yield (Kalscheur et al., 2006).

Another factor that can affect the estimated requirement for RDP is the degree of urea N recycling to the gastro-intestinal tract (GIT) (Lapierre and Lobley, 2001). Several studies have demonstrated that the level of urea N recycling is proportional to N intake and can easily achieve 70% of digestible N (Lapierre et al., 2004; Ouellet et al., 2004). The NRC (1985) and CPM Dairy (Boston et al., 2000) use a quadratic equation to predict the amount of urea reentering the GIT; this equation is based on CP intake (NRC, 1985) but will underestimate recycled urea N by up to 75% (Van Amburgh and Peterson, 2004). The current Dairy NRC (2001) does not have a recycling component, which may partially explain the high RDP requirement.

In addition, endogenous sources of protein were shown to be utilized by ruminal bacteria at the same level as recycled urea N (Ouellet et al., 2002) and this protein would most likely be in the form of peptides. Under in vitro conditions, peptides have been shown to enhance bacterial growth (Chen et al., 1987; Russi et al., 2002; Yang, 2002); however, intraruminal bacterial and protozoal turnover may contribute substantial amounts of peptide N for both bacterial recycling and amino acid supply

(Oldick et al., 2000; Wells and Russell, 1996) and if these sources are currently being underestimated in routine ration balancing, there are possible further reductions to be made in N feeding to dairy cattle.

Broderick (2003) fed diets that ranged from 15.1 to 18.4% CP with modest deficiency in DMI and milk yield. This was consistent with the data of Groff and Wu (2005) suggesting that if we want to formulate diets to which milk yield is sensitive to level of total CP and rumen available N, diet formulation would have to be at or below 15% CP.

There were three main objectives of this study. The first was to evaluate the sensitivity of CPM Dairy predictions for ruminal N balances and CPM Dairy and the Dairy NRC 2001 predictions for MP supply for milk production in dairy cattle fed diets expected to be marginal in total N balance. A second objective was to examine N efficiency in high-producing lactating cattle consuming corn silage diets and fed for an extended period of time to determine if the milk yield and productivity were biologically sustainable on the formulated low CP diets. The third objective was to determine the differences in income over feed cost (IOFC) due to cow responses to the treatment diets.

MATERIALS AND METHODS

Cows and Experimental Design

All experimental procedures were approved by the Cornell University Institutional Animal Care and Use Committee. This experiment was conducted from August 2005 to May 2006. Ninety-three multiparous, early lactation Holstein cows were assigned to three dietary treatments in a randomized complete block design, blocking by average daily milk production over the first 50 d of lactation. Average DIM, daily

milk production over the first 50 d in milk, and age at the start of the study were 80 (± 20) d, 38.4 (± 10.2) kg/d, and 48 (± 12) months, respectively. The cows were housed in a tie-stall barn with rubber mats, bedded with sawdust, and were milked three times per day at 0800, 1600, and 0000. The cows were transitioned onto the treatment diets by providing a 50:50 mixture of the herd and the treatment diets for 7 d, then completely transitioned onto the treatment diets by day 8. Once transitioned to the treatment diets, the study was conducted for 100 d. Five animals were dropped due to non-study related reasons (2 from Diet N and 3 from Diet P) (two cows with chronic mastitis, one cow suffered an aneurism, and two cows had feet and leg injuries) and were not included in the analysis. Four cows per treatment were instrumented with rumen cannulas for rumen sampling during the treatment period.

Diet Formulation

Three diets were formulated in CPM Dairy v.3.0 using library values for all ingredients except corn silage. Each diet consisted of approximately 50% corn silage and 2% wheat straw, with the remainder consisting of an ingredient mix specific to the treatment objectives (Table 1). For the purpose of this paper, these treatments and diets will be identified as Positive control (**P**), Negative control (**N**), and Treatment (**T**). Corn silage was used as the primary forage because it allowed us to more easily control the amount of N coming from the forage in an effort to feed lower protein diets.

All diets were formulated with CPM Dairy V3 at an estimated daily DMI of 22.2 kg for a 625 kg cow producing 36.3 kg milk/d at 3.70% fat and 2.95% true protein. All diets were formulated to be isocaloric on a ME basis at 2.72 Mcal ME/kg. Diet T was designed to be positive for MP balance but negative for ruminal N balance and was formulated at 14.1% CP, with a rumen N balance of negative 39 g/d and an MP

Table 1. Proportional composition of ingredients for Diet P, N, and T.

	% of total ration DM		
Ingredient	**Diet P**	**Diet N**	**Diet T**
Corn silage, processed	46.13	45.24	45.34
Wheat straw, chopped	2.10	2.06	2.06
Soybean hulls	4.19	4.11	4.12
Corn grain, finely ground	11.12	11.31	10.92
Cottonseed, whole with lint	8.39	8.23	8.45
Citrus pulp	5.24	9.26	9.28
Soybean meal (47.5% CP)	5.77	7.81	.....
Barley grain, ground	4.19	8.23	6.18
Expeller soybean meal[1]	6.29	.....	5.15
Animal protein blend[2]	1.05	.....	2.27
Mepron[3]	0.05	.....	0.05
Sugar[4]	2.62	0.82	3.09
Nitroshure[5]	0.38	0.41	0.35
Vitamin pre-mix[6]	0.25	0.24	0.25
Salt	0.50	0.55	0.50
Calcium diphosphate	0.11	0.16	0.29
Calcium sulfate	0.21	0.24	0.25
Magnesium oxide	.....	0.02	0.04
Magnesium sulfate	0.21	0.20	0.25
Limestone	1.20	0.97	0.86
Potassium chloride	.....	0.12	0.31

[1]Soyplus, West Central Cooperative, Ralston, IA
[2]Provaal, Venture Milling, Fulton, NY
[3]Degussa Corp. Parsippany, NJ
[4]Blend of 50% sucrose and 50% confectioner sugar. (Round House Mills, Cortland, NY)
[5]Balchem Corp., New Hampton, NY
[6]Formulated to provide (per kg of DM) 30 g Ca, 250 g Mg, 60 g K, 88 g S, 3.7 g Cl, 8.6 g Fe, 18.6 g Zn, 6 g Cu, 16 g Mn, 100 mg Se, 330 mg Co, 570 mg I, 3022 KIU vitamin A, 1027 KIU vitamin D, 20264 IU vitamin E.

balance of 91 g/d. Given the lower ruminal N balance of Diet T, we anticipated some possible reduction in DMI due to possible potential ruminal N limitation and reduced fiber digestibility (Stokes et al., 1991a). To reduce the rumen available N and increase the MP supply of Diet T compared to Diet N, solvent extracted soybean meal was exchanged for extruded soybean meal (SoyPlus®, West Central Cooperative, Ralston, IA) and a low rumen degradability animal protein blend (ProvAAL, Venture Milling, Fulton, NY) was used. A rumen protected Met source (Mepron, Degussa, Co.) was used to balance the Lys to Met ratio at 3.1:1 and the Lys and Met contents were 6.99 and 2.26% of the predicted MP supply. To provide a more constant availability of ruminal ammonia, an encapsulated urea product (Nitroshure®, Balchem Corp., New Hampton, NY) was used to potentially increase N efficiency by reducing ammonia loss out of the rumen and subsequent urea clearance by the kidney and was included in all diets.

Diet P was designed to represent a positive control, where both the MP balance and the rumen N balance were positive. Thus, to meet these objectives the diet was formulated at 16.3% CP, and predicted to supply a ruminal N balance of 29 g/d and an MP balance of 264 g/d (Table 2) and utilized ingredients common to all three diets. The formulated Lys:Met ratio was 3.1:1 and the Lys and Met contents as a percent of MP were 6.88 and 2.22.

Finally, Diet N was designed to provide adequate ruminal N but be deficient in MP balance. To accomplish this it was formulated at 14.0% CP, with a rumen balance of 27 g/d, but an MP balance of negative 145 g/d. The primary protein ingredients used to meet predicted rumen N requirements were solvent extracted soybean meal and the encapsulated urea product. No consideration was given to Lys or Met balance or ratios. A blend of sugars (50% sucrose, 50% confectioner sugar) (Round House Mills, Cortland, NY) was included in all diets to increase rumen available

Table 2. CPM Dairy V3.0 predicted performance for a 626 kg cow consuming 22.2 kg DMI/d and producing 36.3 kg milk/d at 3.70% fat and 2.95% true protein.

Predicted performance	**Diet P**	**Diet N**	**Diet T**
ME allowable milk, kg/d	40.4	39.4	39.4
ME balance, Mcal/d	4.48	3.43	3.38
MP allowable milk, kg/d	42.1	33.1	38.3
MP balance, g/d	264	-145	91
Peptide and NH_3 balance, g/d	29	27	-39
Peptide and NH_3 balance, % req.	107	107	90
Peptide balance, g/d	18	4	-46
Peptide balance, % req.	109	102	79
MP from bacteria, g/d	1434	1478	1347
MP from RUP, g/d	1248	808	1185

carbohydrates and an anticipated greater demand for rumen N, through increased fermentation (Stokes et al., 1991a).

Feed was provided as a TMR once daily between 0800 and 1000 with target refusals as 10% of the amount fed. The concentrate mix for each treatment was delivered approximately bi-weekly (Round House Mills, Cortland, NY) and included all ingredients except the corn silage, straw, and monensin. Dry matter content for the corn silage and concentrate mix was determined weekly (55ºC in forced air oven for 48 hr) and amounts fed were adjusted accordingly. Feed refusals were measured daily and TMR and orts samples were taken weekly, dried, and analyzed for CP. Daily feed refusals were used to calculate DMI per cow. Milk yield was recorded daily via electronic parlor measurements, and a milk sample was taken for each of the three milkings on one day each week, preserved with 2-bromo-2-nitropropane-1, 3-diol and refrigerated at 36ºC until analysis. Each sample was analyzed separately. Milk composition was based on the weighted average of the individual milk yields and their associated component concentrations. Cows were given bST per label (Posilac, Monsanto Co., St Louis, MO.) and monensin (Elanco Animal Health, Greenfield, IN) was formulated in the diet at an inclusion rate of 300 $mg*cow^{-1}d^{-1}$. Body weights were measured biweekly, and body condition scores were recorded weekly by two individuals whose scores were averaged. Blood samples were collected weekly on the same day before feeding via the coccygeal vein, placed on ice, and centrifuged for plasma removal. Twelve cows (n = 4 per treatment) had rumen fistulas which allowed for rumen measurements.

Milk fat depression (MFD) was observed during the study. To determine one potential source of the MFD (Duffield et al., 2000), approximately half of the cows on each treatment (11, 16, and 15 cows in the N, P, and T treatments, respectively) were maintained on experimental diets after the 100d study period and monensin was

removed from the diets. Milk yields were recorded daily and milk components were determined weekly for four weeks after removal of monensin.

A partial budget analysis was conducted to examine the IOFC from feeding the low CP diets, Diets N and T. The goal of this analysis was to determine if feeding cows either of these diets would result in higher net income over feed cost. If so, it suggests that they may be a feasible management option for high producing dairy cattle, particularly in regions harvesting high quality corn silage. Since total labor and reproductive parameters did not differ among cows fed the three diets, they were assumed to be equivalent among animals fed each diet and were not included in the partial budget analysis. If indeed these or other parameters were truly different, they should be accounted for in the implementation of these types of diets in dairy cattle rations. The data required for the partial budget analysis were the average ration cost over the course of the feeding period, DMI, and the revenue from milk production based on pricing formulae from federal milk pricing regulations using milk components (Agricultural Marketing Service, 2006).

Sample Analysis

Milk samples were analyzed for total fat, protein, lactose, MUN, SCC on either a Foss 4000 or Foss 6000 spectrometer (Dairy One, Ithaca, NY). Plasma samples were assayed for plasma urea N (PUN) using the procedure of Chaney and Marbach (1962).

Ingredient, TMR, concentrate mix, and orts samples were dried in a 55ºC oven, ground with a 2-mm screen using a Wiley Mill, and analyzed for CP (AOAC, 1990), NDF (Mertens, 2002), NDIN, ADF, ADIN, and lignin (Van Soest et al., 1991). Rates of NDF degradation were determined on 6 and 24 h in vitro digestion samples (Van Amburgh et al., 2003). Starch was evaluated using a modification of the AOAC method 996.11 (McCleary et al., 1997). The modification was based on increased

sample size and reagent use to reduce variation among and within samples (Smith et al., unpublished). Samples for corn silage starch degradation were ground through a 4-mm screen using a Wiley Mill and 1 g samples were incubated in Erlenmeyer flasks in Goering and Van Soest (1970) buffer and rumen fluid for 6, 9, 12, and 24 h. Starch recoveries were measured as described previously. Data for rates were fit to a single pool model with a discrete lag (TableCurve, 5.01, Systat Software) to determine a first order rate of degradation for use in CPM Dairy. Corn silage was also analyzed for organic acid and volatile fatty acids (Dairy One, Ithaca, NY).

Rumen fluid samples were taken by obtaining samples of fluid from various locations throughout the rumen, pooling them, and squeezing them through 4 layers of cheesecloth. Rumen pH was taken immediately after sampling approximately 8-10 times over the course of two days using a Fisher Accumet ® model 630 pH meter. Ruminal ammonia was measured using the procedure of Chaney and Marbach (1962) on 2 acidified rumen fluid samples per cow (1 mL 1 N H_2SO_4 to 40 mL rumen fluid resulting in pH < 2) taken approximately 1-2 hours after removal of feed from the four fistulated cows on each treatment diet. Also from those rumen samples, volatile fatty acids were measured by HPLC using crotonic acid as an internal standard (Seigfried et al., 1984).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (2001). Dry matter intake, N intake, milk yield, ECM yield, fat and protein yield, BW, BCS, ruminal VFA, ruminal NH_3, MUN, and PUN were analyzed as repeated measures by week of treatment with diet as the treatment and cow as the subject. Diet by week interactions were tested and were removed from the model when not significant. The following model was used:

$Y_{ijk} = \mu + C_i + T_j + W_k + T_j*W_k + \varepsilon_{ijk}$

where C_i is the effect of the i^{th} cow, T_j is the effect of the j^{th} treatment, W_k is the effect of the k^{th} week, T_j*W_k is the jk^{th} effect of the treatment by week interaction, and ε_{ijk} is random error.

A first order autoregressive covariance structure was used to model the data within cows across the repeated measure of week. This structure was chosen due to its low Bayesian information criteria compared to the simple, unstructured, compound symmetry, and heterogeneous compound symmetry structures (Littell et al., 1996). Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Diet Composition and Dry Matter Intake

Composition of the diets was similar to formulated values, and the formulated protein levels were very similar to what the cows were offered (Table 3). Based on inventory, we planned to feed corn silage from one bunk silo for the entire experimental period. However, due to unforseen spoilage, we fed corn silage from two different bunk silos although the chemical composition and digestibility of the silages was similar (Table 4), so the concentrate mixes and diets remained similar throughout the study. The digestibility of the corn silage was different from the first to the second bunk silo, but no differences in DMI, overall animal performance, or interactions were observed.

Overall, DMI were equal to or greater than previously conducted studies with corn silage as the major forage component (Cabrita et al., 2003; Oba and Allen, 1999; Wattiaux and Karg, 2004b; Weiss and Wyatt, 2006). Cattle consumed, on average, 2.9 kg more DM/d than originally formulated. However, consistent with our initial

Table 3. Chemical composition of Diet P, N, and T.

Fraction	Diet P	Diet N	Diet T
DM, %	48.4	48.8	48.8
CP, % DM	16.2	14.1	14.0
Soluble protein, % CP	29.9	35.6	30.4
RDP[1], % CP	40.5	67.1	55.4
RDP, % DM	9.6	9.5	7.8
NDF, % DM	34.5	34.7	34.8
Lignin, % DM	1.9	1.9	1.9
Starch, % DM	24.6	26.1	25.4
Sugar[2], % DM	5.3	4.8	6.0
Silage acids[3], % DM	2.1	2.0	2.0
Soluble fiber[4], % DM	7.8	8.8	8.3
Ether extract, % DM	4.6	4.4	4.5
Ash, % DM	7.4	7.4	7.5

[1]Rumen degradable protein, calculated by CPM Dairy v3.0 (Boston et al., 2000)
[2,3,4]Calculated from CPM Dairy v3.0 (Boston et al., 2000)

Table 4. Chemical composition of the corn silage.

	Corn silage 1	**Corn silage 2**
DM, % as fed	33.02	29.95
NDF, % DM	43.38	44.23
ADF, % DM	24.20	25.33
Lignin, % DM	2.53	2.48
CP, % DM	7.15	8.19
Starch, % DM	36.78	32.45
24 hr NDFd, % NDF	48.15	47.82
Estimated kd,[a] %/hr	3.9	3.7

[a]Estimated according to the method of Van Amburgh et al., 2003.

Table 5. Least square means of DMI, CP, and NDF intake and milk yield and BW parameters for cows fed Diets P, N, and T.

Performance	Diet P	Diet N	Diet T	SEM	Diet effect	Diet*Week interaction
n	29	29	30			
DMI, kg/d	25.66[a]	25.45[a]	24.21[b]	0.40	0.02	0.0026
CP intake, kg/d	4.18[a]	3.65[b]	3.44[c]	0.06	<0.0001	NS
NDF intake, kg/d	8.94	8.94	8.77	0.14	0.62	0.0034
Milk yield, kg/d	45.00[a]	42.62[b]	43.29[ab]	0.75	0.06	NS
3.5% FCM yield, kg/d	39.08[a]	36.53[b]	36.37[b]	0.82	0.03	NS
Fat %	2.68	2.67	2.54	0.08	0.37	NS
Protein %	2.93	2.92	2.90	0.04	0.85	NS
Lactose %	4.68	4.70	4.62	0.04	0.29	NS
Milk energy yield, Mcal/d[1]	26.97[a]	25.22[b]	25.03[b]	0.55	0.02	NS
Fat yield, kg/d	1.20[a]	1.12[ab]	1.09[b]	0.03	0.09	NS
Protein yield, kg/d	1.32[a]	1.23[b]	1.24[b]	0.02	0.01	NS
Lactose yield, kg/d	2.13	2.00	2.00	0.05	0.08	NS
Body weight, kg	677	644	669	12.20	0.14	0.06
BCS	2.81	2.74	2.80	0.05	0.61	NS
Milk N:Intake N	0.31[c]	0.33[b]	0.36[a]	0.004	<0.0001	0.0011
Feed efficiency, kg 3.5% FCM/kg feed	1.53[a]	1.46[b]	1.51[ab]	0.02	0.01	0.0004

[abc]Values in rows with different superscripts differ $P < 0.05$ as evaluated by means separation procedure in the Mixed procedure of SAS (2001).

[1]Calculated by NRC (2001) equation 2-15.

formulation, the cows offered Diet T consumed approximately 1.3 kg less DM/d ($P < 0.05$) compared to cattle offered the other two diets (Table 5), and there was a linear decrease in CP intake among the treatments (Diet P to T, respectively).

Crude protein levels less than 16% have been implicated in reduced DMI. Roffler et al., (1986) demonstrated a 0.4 kg/d increase in DMI when protein levels were increased from 14 to 17% and Cabrita et al., (2003) demonstrated a 2 kg/d increase in DMI when cows were fed corn silage based diets containing 15.7 versus 14% CP. Similarly, Broderick (2003) showed a linear increase in DMI when dietary CP was increased. In the current study, it is possible that the cattle fed Diet T were moderately deficient in rumen available N and this was reflected in the rumen ammonia measurements (Table 6) that approached the lower limits for adequate fiber digestibility as suggested by Satter and Slyter (1974). The higher than expected DMI suggests that the combination of low CP and low RDP might have been marginal but most likely did not lead to significant effects on fiber digestion; however, we did not measure total tract digestibility on this study. These data suggest that the cows were at a level of both CP and rumen available N to be sensitive to changes in protein source and supply, and this could serve as a reference for future studies and to further understand where offsets exist in the current structure of ration evaluation systems like CPM Dairy.

In the current study, Diets P, N, and T contained 9.5, 9.4, and 7.7% RDP as % DM as predicted by CPM Dairy. Low RDP levels (< 9% of DM) have been implicated in decreased forage digestion and reduced DMI (Broderick, 2003; Dhiman et al., 1993). However, this relationship is not consistent and often depends on the nutrient supply from other dietary components. Several studies with varying RDP values have corresponding changes in total CP supply, potentially obscuring the effect of low RDP (Cabrita et al., 2003; Kalscheur et al., 2006). For example, Reynal and Broderick

(2005) found no effect on DMI or milk yield with a range of 7.7 to 12.5% RDP and overall diet CP levels were maintained above 17% CP. Further, Kalscheur et al., (2006) fed diets that ranged from 6.8 to 11.2% RDP but ranged from 12.3 to 17.1% CP and found no effect on DMI; however, DMI were 4 to 5 kg/d lower than our study, suggesting that the effect of RDP was obscured by the differences in CP level.

Milk Yield and Milk Composition

Average milk yield for all cows at the Cornell Teaching and Research Facility during the study period was approximately 36 kg milk/d at 3.65% fat and 3.0% protein, and that is the level of milk yield the diets were formulated to support. Milk yield over the study period was significantly greater for cows fed Diet P compared to cows fed Diet N; however, cows fed Diet T diet were not different from either treatment ($P > 0.05$) (Table 5). Milk fat, protein, and lactose content did not differ among the three treatments, however, due to the higher milk yield observed in cows fed Diet P, significantly higher milk energy yield, milk protein yield, and 3.5% FCM ($P < 0.05$) were observed for cattle fed this diet (Table 5). The observed milk yields and component yield are similar to or greater than previous studies which used corn silage as the primary forage source (Davidson et al., 2003; Groff and Wu, 2005; Wattiaux and Karg, 2004a). Our data are consistent with other studies demonstrating that as CP content falls below approximately 16%, protein availability, either as MP or rumen available N, become limiting and this has a significant effect on milk yield (Wu and Satter, 2000; Broderick, 2003; Groff and Wu, 2005; Kalscheur et al., 2006).

We anticipated that cows fed Diet T would have adequate MP supply to achieve similar milk yield to cows fed Diet P and would recycle adequate urea N to overcome the predicted ruminal deficiency. This hypothesis is supported based on the lack of difference in milk yield between the two groups of cows fed the treatment diets. Cows

fed Diet N were apparently limited on MP supply, but the degree of limitation was not consistent with the predicted MP balance (Table 2). The 3.5% FCM yield was significantly different between cows fed Diet P and the other two treatment diets; however, this difference appears to be more a consequence of the MFD than the pre-planned treatment effects.

Previous studies comparing corn silage as a primary forage with alfalfa silage have observed decreases in milk fat content by approximately 10 to 18% (Cabrita et al., 2003; Wattiaux and Karg, 2004a). Reductions in milk fat are associated with high levels of polyunsaturated fatty acids and the formation and absorption of trans fatty acid isomers known to affect milk synthesis (Bauman and Griinari, 2003). In the current study, milk fat depression was significant and was most likely exacerbated by the presence of monensin in the diet (Phipps et al. 2000; Duffield et al., 2003).

Low or reduced milk fat content and yield have been observed in cows fed diets high in corn silage, but rarely are they observed to be as low as reported in the current study (Cabrita et al., 2003; Wattiaux and Karg, 2004a). To our knowledge, this study represents the longest controlled period of such a significant MFD. Others have fed protected CLA in an effort to cause MFD and alter energy balance, (Perfield et al., 2002; Bernal-Santos et al., 2003; Castaneda-Gutierrez et al., 2005) for up to 20 wks, but never achieved the MFD observed in this study. This observation does confound our experimental results and interpretation. A possible explanation for the low milk fat content might be due to the presence of high amounts linoleic acid in the ration in combination with monensin. Using library values from CPM Dairy for linoleic acid content based on the measured ether extract content of each feed ingredient, the expected intake of linoleic acid was approximately 500 g/d due to the large proportion of corn based feeds and cottonseed.

Diets high in polyunsaturated fatty acids (PUFA) are more susceptible to MFD. Moderate ruminal pH values and monensin also have been shown to contribute to it and in combination, these two factors may exacerbate the effect (Fellner et al., 1997; Jenkins et al., 2003; Lock et al., 2006). In particular, most bacteria identified thus far to be involved in biohydrogenation are gram positive, which are also those affected by monensin (Fellner et al., 1997). It is possible that the PUFA were partially biohydrogenated in the rumen by bacteria not affected by the ionophore, then not able to become fully biohydrogenated due to the reduction in the microbial population that completes this process (Jenkins et al., 2003), thus allowing intermediates that can induce MFD to accumulate and be absorbed (Lock et al., 2006).

To determine if monensin was partially responsible for MFD, an evaluation was made only with cows that continued to be fed the study diet without monensin after the 100 d study period. There were 42 cows retained on study diets without monensin among all treatments, and the mean milk fat % increase was approximately 22%, from 2.67% to 3.26% ($P < 0.0001$), values much closer to the general herd milk fat percentage. This increase occurred within two weeks after the removal of monensin and demonstrates that the dietary treatments per se had a very small effect on milk composition and potential yield of milk components.

Milk and Plasma Urea Nitrogen

Typical MUN or PUN values for high producing cows range from 10 to 15 mg/dl (Wattiaux and Karg, 2004a; Groff and Wu, 2005). Plasma urea nitrogen values for cows consuming Diet P were within that range and were significantly higher than values for the cows consuming Diets N and T and reflected the diets consumed. An interesting observation was that MUN tended to be greater than PUN. Since MUN is a reflection of PUN, having higher concentrations of urea in the milk than in the

Table 6. Plasma and milk urea nitrogen, ruminal pH, and ammonia levels and volatile fatty acid concentrations for cows fed Diets P, N, and T.

Performance	Diet P	Diet N	Diet T	SEM	Diet effect
PUN, mg/dl	11.31[a]	8.40[b]	7.13[c]	0.14	<0.001
MUN, mg/dl	11.11[a]	8.74[b]	8.43[b]	0.20	<0.001
Ruminal pH	5.95[a]	6.06[a]	5.93[a]	0.14	0.66
Ruminal NH_3, mg/dl	6.58[a]	8.32[a]	5.84[a]	2.38	0.66
Total VFA, mM	118.15[a]	119.34[a]	144.05[a]	13.94	0.18
Lactate, mM	0.37[a]	0.04[a]	0.75[a]	0.63	0.60
Formic acid, mM	7.87[a]	10.07[a]	8.99[a]	1.71	0.56
Acetic acid, mM	65.16[a]	64.95[a]	70.87[a]	5.37	0.53
Propionic acid, mM	30.52[a]	29.71[a]	44.88[a]	7.60	0.15
Acetate:propionate	2.37[a]	2.43[a]	1.93[a]	0.32	0.32
Isobutyric acid, mM	0.85[a]	0.73[a]	1.24[a]	0.28	0.25
Butyric acid, mM	10.61[a]	10.05[a]	14.20[a]	1.72	0.08
Isovaleric acid, mM	1.79[a]	2.39[a]	1.61[a]	0.32	0.12
Valeric acid, mM	0.99[a]	1.40[a]	1.50[a]	0.38	0.45

[abc]Values in rows with different superscripts differ $P < 0.05$ as evaluated by pdiff contrast in the Mixed procedure of SAS (2001).

plasma suggest there are some potential problems with calibration methods on the scanning equipment and that our samples were outside the normal calibration range (Kohn et al., 2004).

The mean PUN value for cows consuming Diet T was much lower than most studies published on lactating dairy cattle, suggesting that these animals were functioning on a very limited supply of N. Both PUN and MUN have been used to evaluate N status in the animal, to indicate N consumed in excess of that required for productive functions (Kohn et al., 2002; Nennich et al., 2006). These values from cows fed Diet N and T suggest little excess N was available. The PUN levels also suggest that ureagenesis was more dependent on total protein intake than rumen degradability, since cows fed Diet N were predicted to be positive on ruminal N balance. However, PUN values were similar for cows fed both Diets N and T which were also similar in total CP intake. Further, if recycled urea N is a function of total pool size as has been suggested (Lapierre and Lobley, 2001), then cows consuming Diet P should have greater urea N reentry into the GIT, but with potentially less microbial efficiency (Marini and Van Amburgh, 2003).

Rumen Measurements

Ruminal NH_3 concentrations did not accurately reflect predicted ruminal peptide and NH_3 balances (Tables 6 and 7), as the mean ruminal NH_3 concentration for Diet N was higher than that for Diet P, but the predicted balance was nearly identical. Nor were there any significant differences in ruminal NH_3 concentration due to diet effects, suggesting that predicted balances may not have been indicative of actual ruminal NH_3 status. It has been suggested that concentrations below 5 mg/dl represent concentrations at which microbial protein growth is potentially limited (Satter and Slyter, 1974), and the ruminal NH_3 concentration can be dependent on the amount of

fermentable energy (Oba and Allen, 2003; Shabi et al., 1998), making it difficult to define a specific concentration at which a deficiency is declared. In cows producing approximately 33 kg milk/d, no decrease in production was observed with ruminal NH_3 concentrations of 7 to 8 mg/dl (Davidson et al., 2003). However, Reynal and Broderick (2003) reported decreased milk yield and DMI in cows with ruminal NH_3 concentrations of 8 mg/dl compared to 9 mg/dl and above. In the current study, cows fed Diet P had a ruminal NH_3 concentration of 6.6 mg/dl, but apparently this had no negative effects on DMI or milk yield. Further, it is hard to determine from these data if in fact cows fed Diet T were ruminally N deficient, but as the ruminal NH_3 concentration was very similar as that observed in Diet P, it is likely that this variable had little or no impact on the lower DMI and 3.5% FCM yield directly. Numerical differences in NH_3 concentration in the current study may have been due to slight differences in fermentable energy, particularly the addition of sugar in the diet and the differences in total CP intake.

Given the lack of difference in ruminal NH_3 between cows fed Diets P and T, but significant differences in milk yield, we suspect that the primary difference was simply the amount of MP available to the animals fed Diet P. Due to the significant decrease in DMI of cows fed Diet T, there was a 0.74 kg difference in daily CP intake, and that difference in CP intake was most likely responsible for the difference in milk yield, although not significantly different from cows fed Diet P.

Volatile fatty acid concentrations were not different among treatments, similar to data from Ipharraguerre et al. (2005) except for the branched chain volatile fatty acids (BCVFA). They observed a significant difference in BCVFA in diets ranging from 13.9 to approximately 18% CP, however they did not detect a difference in cows fed diets from 16.1 to 13.9% CP, consistent with data from this study. This does not mean that BCVFA were not potentially limiting for cellulolytic bacteria to digest fiber

(Bryant, 1973; Dehority et al., 1967). However, it is difficult to conclude whether the cows in the current study were deficient since DMI and milk yield was, despite the differences among treatment, relatively high.

Body Weights, Condition Scores, and Reproductive Efficiency

Cows fed Diet N had a numerically lower mean BW, but this difference was not significant and was apparently a random event since BW was not used as a criteria for treatment assignment. Cows on all treatments increased in BW over the 100 d experiment; the overall BW gain was 0.40, 0.34, and 0.30 kg/d for the P, N, and T treatments, respectively, and was not significantly different (Table 5). These BW gains were similar to those reported by Davidson et al., (2003) for cows fed corn silage diets in early lactation. However, their diets contained 16.5% CP on average, suggesting that cows in the current study demonstrated that reductions in CP supply are possible without mobilizing body protein. Body condition score was similar among treatment and averaged approximately 2.8 throughout the study period with very little change in BCS over the 100 d study period. Since the days of study encompassed the period of breeding and confirmation of pregnancy, we evaluated reproductive parameters as a consequence of the study design. Reproductive efficiency was similar among all treatments and average services per conception were 2.8, 3.0, and 3.0 and days to confirmed pregnancy were 142, 155, and 148 for the P, N, and T diets, respectively, and were not different among treatments.

Model Predictions

Post-treatment, the feed chemical composition of all the ingredients were re-inputted into CPM Dairy and the Dairy NRC (2001) along with all of the specific animal production characteristics in order to evaluate the predicted milk yield (Table

7). CPM Dairy allows the user to account for the effects of BW gain on nutrient utilization; thus two evaluations were conducted, the first with a fixed BW and the second with BW gain over the treatment period included in the predictions.

Predicted Rumen N Balances

After accounting for the actual feed chemical composition and mean animal values per treatment, CPM Dairy predicted ruminal peptide and NH_3 balances remained positive for both Diet N and P at 48 and 56 g N/d, but were marginally negative for Diet T at -22 g N/d (or 95% of the recommended level) (Table 7). These predictions were not consistent with the measured ruminal NH_3 values since rumen NH_3 was similar for cows fed Diets P and T and suggest that the predictions were over-estimating the rumen N balance, despite the known under-prediction of recycled urea N. One possible explanation for a part of this disparity could be the passage of soluble protein out of the rumen in the fluid phase which CPM Dairy is currently insensitive to. Faster passage of the soluble proteins out of the rumen would have the obvious effect of decreasing rumen available N. Consistent with this, Ipharraguerre et al., (2005) and Reynal and Broderick (2005) demonstrated that small peptides were passing out of the rumen in the fluid phase at a rate faster than the solids passage rate in CPM Dairy. Therefore, the model would be more sensitive to the levels of CP and rumen available N supplied in our treatment diets.

The CPM Dairy model uses an equation from the 1985 NRC to predict urea recycled into the rumen ($U = 121.7-12.01*CP+0.3235*CP^2$), where U = urea N recycled (percent of N intake) and CP = dietary CP intake (percent of DM). Use of this equation results in a quadratic equation with a minimum amount of dietary N recycled at 18.6% CP (NRC, 1985). The predicted amount of recycled urea N for the

Table 7. CPM Dairy V3.0 predicted performance using mean values per treatment diet for cow (BW, DMI, milk component composition) and the actual dietary chemical composition. Body weights were either assumed to be constant or the actual changes over the study period were included in model predictions.

Actual Milk Yields and Predictions	**Diet P**	**Diet N**	**Diet T**
Actual milk yield, kg/d	45.00	42.62	43.29
3.5% FCM yield, kg/d	39.08	36.53	36.37
CPM Dairy Predition			
BW constant			
ME allowable milk, kg/d	53.9	53.3	50.8
ME balance, Mcal/d	8.5	10.1	7.0
MP allowable milk, kg/d	46.9	37.5	42.8
MP balance, g/d	87	-226	-23
Actual BW changes included			
ME allowable milk, kg/d	48.9	49.2	47
ME balance, Mcal/d	3.7	6.2	3.4
MP allowable milk, kg/d	43.3	34.6	40.0
MP balance, g/d	-71	-362	-146
Peptide and NH_3 balance, g/d	56	48	-22
Peptide and NH_3 balance, % requirement	113	111	95
Peptide balance, g/d	17	-3	-51
Peptide balance, % requirement	107	99	77
MP from bacteria, g/d	1556	1606	1465
MP from RUP, g/d	1443	960	1288
NRC 2001 Prediction			
NEl allowable milk, kg/d	49.4	47.4	45.3
MP allowable milk, kg/d	44.2	35.7	31.6

current study diets was between 80 and 97 g N/d. Recent studies have demonstrated that 39 to 76% of the intake N is recycled back to the GIT in the form of urea (Lapierre et al., 2004; Ouellet et al., 2004). If the minimal level suggested by these studies is recycled, calculated as the percent of intake N, cows on Diet T would recycle approximately 200 g N/d, which would make the rumen NH_3 balance positive. Since the ruminal NH_3 concentrations nor milk yield were not significantly lower for Diet T than Diet P, this suggests that cows consuming Diet T were not deficient in ruminal N despite the lower ruminal NH_3 concentrations.

Predicted ME and MP allowable milk

The re-evaluation of predicted ME allowable milk when BW was held constant suggests that compared to energy intake, the cows on all treatments were in positive energy balance by approximately 8 Mcal ME per day. This large discrepancy in ME balance is a function of the MFD, but represents a significant difference in apparent energy availability since BW gain and BCS changes were not reflected by the calculated ME balance. Inputting the daily BW change associated with each animal on study improved the predicted ME balance and reduced the overprediction by approximately 50%. This difference in ME allowable milk compared to the actual milk yield suggests there is still an apparent bias in the prediction of energy allowable milk. This apparent bias might be due to the presence of the trans fatty acid isomers responsible for MFD (de Veth et al., 2006). Cows fed a protected form of CLA showed differences in nutrient partitioning, but the study design was in 16 d blocks, a duration adequate to observe changes in milk composition, but not changes in BW or BCS (de Veth et el., 2006). Due to the duration of the current study and the severity of MFD, we might have observations more related to actual changes in BW or BCS due to a reduction in milk fat synthesis.

The net energy allowable milk predictions of the Dairy NRC (2001) demonstrated an overprediction of energy allowable milk for all treatments, and the overprediction was similar to the CPM Dairy predictions after accounting for BW changes. Under conditions of no BW change, the CPM Dairy predicted MP allowable milk was within 1 kg/d for cows fed Diets P and T and underestimated by approximately 4 kg/d for cows fed Diet N. When BW changes were accounted for, the MP allowable milk was underpredicted from 1.7 to almost 8 kg/d of actual milk yield. This strongly suggests that there are offsets in MP supply currently not being accounted for in CPM Dairy and that the model needs refinement in order to be sensitive to these changes. The most significant observation is the 200 to 300 g/d difference in predicted MP balance among the diets and the relative insensitivity of the cows to the dietary treatments.

An aspect of the MP prediction in CPM Dairy that could be reducing the sensitivity of the model to MP supply predictions are the current pool sizes and rates of degradation for the protein pools. Lanzas et al. (2006) recently evaluated the sensitivity of the Cornell Net Carbohydrate and Protein System (Fox et al., 2004) to pool size calculations and rates of degradation and suggested that modifications could be made to reduce the number of protein pools (collapsing the B2 and B3 pools for example) and alter the rates of degradation to improve the sensitivity of the model to predictions in MP supply. CPM Dairy and the CNCPS share the same feed characterization scheme (Sniffen et al., 1992), thus any factors affecting the CNCPS will have an impact on the predictions of CPM Dairy.

Several studies have demonstrated that predicted milk yield according to NRC (2001) may be oversensitive to total protein and RDP supply. No effect of protein supply on milk or milk component yield was observed in cows fed at 85% and 115% of predicted MP supply (Weiss and Wyatt, 2006). Further, a 13 kg/d difference in milk yield due to MP (89 to 114% of required) and RDP (68 to 111% of required)

supply resulted in only a 2 kg/d difference (Kalscheur et al., 2006). These data are consistent with the current experiment where the NRC (2001) underpredicted MP allowable milk by 1 to almost 12 kg/d. The strong reliance on RDP to meet the microbial protein requirements while underestimating the supply from rumen underdegraded protein has significant consequences on the predicted MP allowable milk for cows fed Diet T compared to Diet P.

There are several other potential N offsets and transactions not properly accounted for in CPM Dairy and the Dairy NRC (2001). For example, endogenous secretions supply peptides and AA, which can be efficiently utilized for microbial growth and MP supply (Lapierre and Lobley, 2001; Ouellet et al., 2002). The NRC 2001 accounts for the reuse of these proteins, using the equation [endogenous metabolizable CP = 4.72*(Total DM fed)], which would supply approximately 120 g MP/d for cows in the current study. If this value was included into the CPM Dairy V3.0 predictions, the MP balances would increase to -108, 208, and 108 g MP/d for the N, P, and T diets, respectively. Perhaps this partially explains the higher than predicted performance by cows fed Diet N in particular, but it must also be recognized that any endogenous recycling would theoretically be accounted for in the maintenance requirement. This would suggest a maintenance protein and energy cost to endogenous protein synthesis, which is calculated in CPM Dairy partially as a function of the indigestible dry matter - a calculation not substantiated by the data of Ouellet et al., (2002) - and a substantial reabsorption of that protein in support of other productive functions.

Another possible factor not included but potentially influencing results is microbial turnover, which has the potential to present large amounts of peptides and AA into the ruminal fluid for subsequent microbial utilization. Currently, neither CPM Dairy nor NRC 2001 directly account for intraruminal N recycling. Wells and Russell (1996) demonstrated, based on in vitro growth rates and theoretical maximum growth yield,

that intra-ruminal recycling cannot exceed 50%, and is most likely between 30 and 45%. Much of the turnover may be due to protozoal predation of bacteria. In faunated sheep, bacterial N recycling was 49% of the flux through the bacterial N pool and only 33% in defaunated animals (Koenig et al., 2000). CPM Dairy accounts for turnover by decreasing the theoretical yield by 20%. However, this representation is not entirely accurate, especially if microbial growth is N deficient, as N is reintroduced to the system with microbial lysis. A potential offset to the reduced theoretical yield is the emerging data suggesting that protozoa contribute from 5 to 15% of the microbial protein flows in lactating dairy cattle (Sylvester et al., 2004). In theory, the MP supply from protozoa should indirectly be accounted for in the predictions of CPM Dairy since MP from bacteria is generally greater than 50% of the total MP supply, consistent with several other data sets, especially those data sets that used the omasal sampling technique instead of duodenal cannulas. Protozoa have a different purine to N ratio than bacteria and are more sensitive to acid hydrolysis in the abomasum.

Predicted N Excretion

The CPM Dairy predictions for urinary and fecal N excretion indicated a significant difference in urinary N output in cows fed Diets N and T with no differences among treatments in fecal N output (Table 8). Among treatments, the values are low compared to other studies with high producing dairy cows (Broderick, 2003; Jonker et al., 2002; Wattiaux and Karg, 2004b) and suggest that if the moderate milk reduction could be offset at some intermediate dietary CP level, the impact of the dairy cow on the environment would be greatly reduced. To compare the prediction of urinary N excretion by CPM Dairy, the equation of Kauffman and St. Pierre (2001) (urinary N = 0.0259*MUN*BW) was used, and the predicted urinary N by this

Table 8. CPM Dairy V3.0 predicted nitrogen excretion based on mean performance values. Body weight change included.

Excretion route	Diet P	Diet N	Diet T
CPM Dairy v3.0 predictions			
Urinary N, g/d	172	94	76
Urinary N, % of total N excretion	40	26	23
Fecal N, g/d	263	264	249
Fecal N, % of total N excretion	60	73	77
Kauffman and St. Pierre, 2001			
Urinary N, g/d	196	154	149

equation was 196, 154, ad 149 g for Diets P, N, and T, respectively, which was similar for cows fed Diet P, but 60 to 70 g/d greater than those generated by the model. Previous comparisons have indicated that the predictions from the equation of Kauffman and St. Pierre (2001) are similar to measured values from cows fed more traditional diets. It is possible that the cows on our study fed Diets N and T were outside the range used to develop the equation, which would help explain the disparity. The milk N:intake N ratio was significantly highest for Diet T, followed by Diet N, and Diet P resulting in the lowest ratio. These efficiencies are similar to that observed in diets whose main forage is corn silage (Wattiaux and Karg, 2004b; Weiss and Wyatt, 2006).

Impact of Reducing Protein Feeding on Net Income Over Feed Cost

Ration costs were calculated based on the average cost of each ingredient over the course of the feeding period. The estimated value of corn silage was $30/tonne based on chemical composition. The cost per tonne (T) of feed are shown in Table 9. Diet N cost approximately 13% less per T than the other two diets, and Diet T cost the most per T. However, due to the difference in mean DMI among the treatments, cows consuming Diet P cost the most to feed per day, whereas cows fed Diet N and T cost 0.52 and $0.19/d less to feed. The average 2006 NASS dairy product prices for butter, non-fat dairy milk, cheese, and dry whey and the utilization of milk in different regulated milk classes (Agricultural Marketing Service, 2006) was used to calculate a weighted-average farm price (the blend price) at actual test for the mean production by cows on each diet.

Due to the low milk components and slightly lower total milk production by cows fed Diets N and T, cows given Diet P produced approximately $0.62/d more in milk revenue. Because of this, the net income over feed cost was $0.11/d and $0.43/d

lower for cows fed Diets N and T, respectively. Over the 100d experimental period, cows consuming Diets N and T made approximately $11 and $43 less in net income over feed costs than cows consuming Diet P. For the average dairy farm in New York with approximately 104 cows (New York State Dept. of Agric. and Markets, 2005), this translates into $1,144 and $4,472 less IOFC for Diets N and T compared to Diet P over a 100d mid-lactation period. Because milk prices were lower for the 2006 data than for most recent years, the reported differences in IOFC are smaller than they would when milk prices are higher. Because ration costs were not a consideration in the experimental design, it may be possible to reduce ration costs yet maintain high performance by cows by feeding a diet similar to those in this experiment.

Table 9. Feed cost, milk revenue, and net income over feed cost for cows fed Diets P, N, and T.

	Diet P	**Diet N**	**Diet T**
Feed cost ($/T)	170.14	151.39	172.56
DMI (kg/cow/d)	25.66	25.45	24.21
Feed cost per animal per day[1] ($/cow/d)	4.37	3.85	4.18
Feed cost relative to Diet P ($/cow/d)	0.00	-0.52	-0.19
Blend price at actual test[2] ($/kg)	0.2603	0.2598	0.2561
Milk yield (kg/cow/d)	45.00	42.62	43.29
Milk revenue ($/cow/d)	11.71	11.08	11.09
Net income over feed cost ($/cow/d)	7.34	7.23	6.91
Net income over feed cost relative to Diet P ($/cow/d)	0.00	-0.11	-0.43

[1]Values may not be statistically different ($P > 0.05$), as mean values for DMI, milk yield, and milk components were used in calculations without inclusion of variance.
[2]The blend price is a weighted-average farm-level price based on the utilization of milk in one of four use classes for Northeast Marketing Order. The calculation assumes a Class I differential of $2.50 representing milk received at Cortland, NY. The utilization of milk in the classes is for 2006 (Agricultural Marketing Service, 2006) and consists of 46.49% Class I, 19.74% Class II, 22.38% Class III, 11.39% Class IV.

REFERENCES

Agricultural Marketing Service. 2006. Federal Milk Order Market Statistics - 2006 Annual Summary.

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists International. Arlington, VA.

Bauman, D. E. and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23:203-227.

Bernal-Santos, G., J. W. Perfield, D. M. Barbano, D. E. Bauman, and T. R. Overton. 2003. Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. J Dairy Sci 86:3218-3228.

Borsting, C. F., T. Kristensen, L. Misciattelli, T. Hvelplund, and M. R. Weisbjerg. 2003. Reducing nitrogen surplus from dairy farms. Effects of feeding and management. Livestock Prod.Sci. 83:165-178.

Boston, R., D. Fox, C. Sniffen, E. Janczewski, R. Munson, and W. Chalupa. 2000. The conversion of a scientific model describing dairy cow nutrition and production to an industry tool: The CPM-Dairy Project. Pages 361-377 in Modeling Nutrition of Farm Animals. CAB International, UK.

Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370-1381.

Bryant, M. P. 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. Fed. Proc. 32:1809-1813.

Cabrita, A. R. J., A. J. M. Fonseca, R. J. Dewhurts, C. V. P. Sampaio, M. F. S. Miranda, G. N. S. Sousa, I. M. F. Miranda, and E. Gomes. 2003. Nitrogen supplementation of corn silages. 1. Effects on feed intake and milk production of dairy cows. J.Dairy Sci. 86:4008-4019.

Castaneda-Gutierrez, E., T. R. Overton, W. R. Butler, and D. E. Bauman. 2005. Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. J Dairy Sci 88:1078-1089.

Chaney, A. L. and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132.

Chen, G., J. B. Russell, and C. J. Sniffen. 1987. A procedure for measuring peptides in rumen fluid and evidence that peptide uptake can be a rate-limiting step in ruminal protein degradation. J. Dairy Sci. 70:1211-1219.

Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304-2323.

Davidson, S., B. A. Hopkins, D. E. Diaz, S. M. Bolt, C. Brownie, V. Fellner, and L. W. Whitlow. 2003. Effects of amounts and degradability of dietary protein on lactation, nitrogen utilization, and excretion in early lactation Holstein cows. J. Dairy Sci. 86:1681-1689.

Dehority, B. A., H. W. Scott, and P. Kowaluk. 1967. Volatile fatty acid requirements of cellulolytic rumen bacteria. J. Bacteriol. 94:537-543.

de Veth, M. J., E. Castaneda-Gutierrez, D. A. Dwyer, A. M. Pfeiffer, D. E. Putnam, and D. E. Bauman. 2006. Response to conjugated linoleic acid in dairy cows differing in energy and protein status. J Dairy Sci 89:4620-4631.

Dhiman, T. R., C. Cadorniga, and L. D. Satter. 1993. Protein and energy supplementation of high alfalfa silage diets during early lactation. J.Dairy Sci. 76, 1945-1959.

Duffield, T. F. and R. N. Bagg. 2000. Use of ionophores in lactating dairy cattle: a review. Can. Vet. J. 41:388-394.

Fellner, V., F. D. Sauer, and J. K. Kramer. 1997. Effect of nigericin, monensin, and tetronasin on biohydrogenation in continuous flow-through ruminal fermenters. J. Dairy Sci. 80:921-928.

Firkins, J. L., A. N. Hristov, M. B. Hall, G. A. Varga, and N. R. St Pierre. 2006. Integration of ruminal metabolism in dairy cattle. J. Dairy Sci. 89(Suppl 1):E31-E51.

Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric.Handbook No.379. ARS-USDA, Washington, DC.

Groff, E. B. and Z. Wu. 2005. Milk production and nitrogen excretion of dairy cows fed different amounts of protein and varying proportions of alfalfa and corn silage. J Dairy Sci 88:3619-3632.

Ipharraguerre, I. R., J. H. Clark, and D. E. Freeman. 2005. Varying protein and starch in the diet of dairy cows. I. Effects on ruminal fermentation and intestinal supply of nutrients. J. Dairy Sci. 88:2537-2555.

Jenkins, T. C., V. Fellner, and R. K. McGuffey. 2003. Monensin by fat interactions on trans fatty acids in cultures of mixed ruminal microorganisms grown in continuous fermentors fed corn or barley. J. Dairy Sci. 86:324-330.

Jonker, J. S., R. A. Kohn, and J. High. 2002. Dairy herd management practices that impact nitrogen utilization efficiency. J. Dairy Sci. 85:1218-1226.

Kalscheur, K. F., R. L. Baldwin, B. P. Glenn, and R. A. Kohn. 2006. Milk production of dairy cows fed differing concentrations of rumen-degraded protein. J. Dairy Sci. 89:249-259.

Kauffman, A. J. and N. R. St Pierre. 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. J. Dairy Sci. 84:2284-2294.

Koenig, K. M., C. J. Newbold, F. M. McIntosh, and L. M. Rode. 2000. Effects of protozoa on bacterial nitrogen recycling in the rumen. J. Anim Sci. 78:2431-2445.

Kohn, R. A., K. R. French, and E. Russek-Cohen. 2004. A comparison of instruments and laboratories used to measure milk urea nitrogen in bulk-tank milk samples. J Dairy Sci 87:1848-1853.

Kohn, R. A., K. F. Kalscheur, and E. Russek-Cohen. 2002. Evaluation of models to estimate urinary nitrogen and expected milk urea nitrogen. J. Dairy Sci. 85:227-233.

Lanzas, C., L. O. Tedeschi, S. Seo, and D. G. Fox. 2007. Evaluation of protein fractionation systems used in formulating rations for dairy cattle. J Dairy Sci 90:507-521.

Lapierre, H. and G. E. Lobley. 2001. Nitrogen recycling in the ruminant: a review. J.Dairy Sci. 84(E. Suppl.):E223-E236.

Lapierre, H., D. R. Ouellet, R. Berthiaume, C. Girard, P. Dubreuil, M. Babkine, and G. E. Lobley. 2004. Effect of urea supplementation on urea kinetics and splanchnic flux of amino acids in dairy cows. J.Anim.Feed Sci. 13(Suppl. 1),:319-322.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS System for Mixed Models. SAS Inst. Inc., Cary, NC.

Lock, A. L., T. R. Overton, K. J. Harvatine, J. Giesy, and D. E. Bauman. 2006. Milk fat depression: Impact of dietary components and their interaction during rumen fermentation. Pages 75-86 in Cornell Nutr. Conf. Proc., Syracuse, NY.

Marini, J. C. and M. E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. J. Anim Sci. 81:545-552.

McCleary, B. V., T. S. Gibson, and D. C. Mugford. 1997. Measurement of total starch in cereal products by amyloglucosidase - alpha-amylase method. J.Assoc.Off.Anal.Chem 80:571-579.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J AOAC Int. 85:1217-1240.

The National Academies Press. 2003. Air emissions from animal feeding operations: Current knowledge, future needs. Washington, D.C.

National Research Council. 1985. Ruminant Nitrogen Usage. Washington, DC, National Academy Press.

National Research Council. 1989. Nutritional Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci, Washington, DC.

National Research Council. 2001. Nutritional Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci, Washington, DC.

Nennich, T. D., J. H. Harrison, L. M. VanWieringen, N. R. St Pierre, R. L. Kincaid, M. A. Wattiaux, D. L. Davidson, and E. Block. 2006. Prediction and evaluation of urine and urinary nitrogen and mineral excretion from dairy cattle. J. Dairy Sci. 89:353-364.

New York State Department of Agriculture and Markets. 2005. New York State Dairy Statistics 2005 Annual Summary.

Noftsger, S. and N. R. St Pierre. 2003. Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. J. Dairy Sci. 86:958-969.

Oba, M. and M. S. Allen. 1999. Effects of brown midrib 3 mutation in corn silage on dry matter intake and productivity of high yielding dairy cows. J. Dairy Sci. 82:135-142.

Oba, M. and M. S. Allen. 2003. Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. J. Dairy Sci. 86:195-207.

Oldick, B. S., J. L. Firkins, and R. A. Kohn. 2000. Compartmental modeling with nitrogen-15 to determine effects of degree of fat saturation on intraruminal N recycling. J. Anim Sci. 78:2421-2430.

Ouellet, D. R., R. Berthiaume, G. E. Lobley, R. Martineau, and H. Lapierre. 2004. Effects of sun-curing, formic acid-treatment or microbial inoculation of timothy on urea metabolism in lactating dairy cows. J.Anim.Feed Sci. 13(Suppl. 1):323-326.

Ouellet, D. R., M. Demers, G. Zuur, G. E. Lobley, J. R. Seoane, J. V. Nolan, and H. Lapierre. 2002. Effect of dietary fiber on endogenous nitrogen flows in lactating dairy cows. J. Dairy Sci. 85:3013-3025.

Perfield, J. W. and D. E. Bauman. 2005. Current theories and recent advances in the biology of milk fat depression. Pages 95-106 in Cornell Nutrition Conference Proc., Syracuse, NY.

Perfield, J. W., G. Bernal-Santos, T. R. Overton, and D. E. Bauman. 2002. Effects of dietary supplementation of rumen-protected conjugated linoleic acid in dairy cows during established lactation. J. Dairy Sci. 85:2609-2617.

Phipps, R. H., J. I. D. Wilkinson, L. J. Jonker, M. Tarrant, A. K. Jones, and A. Hodge. 2000. Effect of monensin on milk production of Holstein-Friesian dairy cows. J.Dairy Sci. 83:2789-2794.

Reynal, S. M. and G. A. Broderick. 2003. Effects of feeding dairy cows protein supplements of varying ruminal degradability. J. Dairy Sci. 86:835-843.

Reynal, S. M. and G. A. Broderick. 2005. Effect of dietary level of rumen-degraded protein on production and nitrogen metabolism in lactating dairy cows. J. Dairy Sci. 88:4045-4064.

Roffler, R. E., J. E. Wray, and L. D. Satter. 1986. Production responses in early lactation to additions of soybean meal to diets containing predominantly corn silage. J. Dairy Sci. 69:1055-1062.

Ruiz, R., L. O. Tedeschl, J. C. Marini, D. G. Fox, A. N. Pell, G. Jarvis, and J. B. Russell. 2002. The effect of a ruminal nitrogen (N) deficiency in dairy cows: evaluation of the cornell net carbohydrate and protein system ruminal N deficiency adjustment. J. Dairy Sci. 85:2986-2999.

Russi, J. P., R. J. Wallace, and C. J. Newbold. 2002. Influence of the pattern of peptide supply on microbial activity in the rumen simulating fermenter (RUSITEC). Br. J. Nutr. 88:73-80.

SAS Institute. 2001. SAS User's Guide: Statistics. Version 8 ed. Cary, NC, SAS Inst. Inc.

Satter, L. D. and L. L. Slyter. 1974. Effect of ammonia concentration of rumen microbial protein production in vitro. Br. J. Nutr. 32:199-208.

Seigfried, V. R., H. Ruckemann, and G. Stumpf. 1984. Eine HPCL-Mthode zur Bestimmung organisher Sauren in Silagen. Landwirtsch.Forschung 37(3-4): 298-304.

Shabi, Z., A. Arieli, I. Bruckental, Y. Aharoni, S. Zamwel, A. Bor, and H. Tagari. 1998. Effect of the synchronization of the degradation of dietary crude protein and organic matter and feeding frequency on ruminal fermentation and flow of digesta in the abomasum of dairy cows. J. Dairy Sci. 81:1991-2000.

Smith, S. E. 2000. An improved exzymatic method for the determination of percent total starch in animal feeds, including both cereals and forages. Honor Thesis. Cornell University.

Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim Sci. 70:3562-3577.

Stokes, S. R., W. H. Hoover, T. K. Miller, and R. Blauweikel. 1991a. Ruminal digestion and microbial utilization of diets varying in type of carbohydrate and protein. J. Dairy Sci. 74:871-881.

Stokes, S. R., W. H. Hoover, T. K. Miller, and R. P. Manski. 1991b. Impact of carbohydrate and protein levels on bacterial metabolism in continuous culture. J. Dairy Sci. 74:860-870.

Sylvester, J. T., S. K. Karnati, Z. Yu, C. J. Newbold, and J. L. Firkins. 2005. Evaluation of a real-time PCR assay quantifying the ruminal pool size and duodenal flow of protozoal nitrogen. J. Dairy Sci. 88:2083-2095.

Van Amburgh, M., P. J. Van Soest, J. B. Robertson, and W. F. Knaus. 2003. Corn silage neutral detergent fiber: refining a mathematical approach for in vitro rates of digestion. Pages 99-108 in Cornell Nutrition Conference Proc., Syracuse, NY.

Van Amburgh, M. E. and E. B. Peterson. 2004. How much nitrogen can a cow recycle? Pages 179-188 in Cornell Nutrition Conference Proc., Syracuse, NY.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wattiaux, M. A. and K. L. Karg. 2004a. Protein level for alfalfa and corn silage-based diets: I. Lactational response and milk urea nitrogen. J. Dairy Sci. 87:3480-3491.

Wattiaux, M. A. and K. L. Karg. 2004b. Protein level for alfalfa and corn silage-based diets: II. Nitrogen balance and manure characteristics. J. Dairy Sci. 87:3492-3502.

Weiss, W. P. and D. J. Wyatt. 2006. Effect of corn silage hybrid and metabolizable protein supply on nitrogen metabolism of lactating dairy cows. J. Dairy Sci. 89:1644-1653.

Wells, J. E. and J. B. Russell. 1996. Why do many ruminal bacteria die and lyse so quickly? J. Dairy Sci. 79:1487-1495.

Wu, Z. and L. D. Satter. 2000. Milk production during the complete lactation of dairy cows fed diets containing different amounts of protein. J. Dairy Sci. 83:1042-1051.

Yang, C. M. 2002. Response of forage fiber degradation by ruminal microorganisms to branched-chain volatile fatty acids, amino acids, and dipeptides. J. Dairy Sci. 85:1183-1190.

CHAPTER THREE: CONCLUSION

Dairy cattle consuming corn silage based diets at 14.0 and 14.1% CP consumed between 24.2 and 25.5 kg DM/d and produced 42.6 to 43.3 kg milk/d on average. These high levels of production with lower dietary CP concentrations than typically fed to dairy cattle suggest that such reductions in CP intake are possible without detrimental effects on cow performance. Cows fed to achieve a positive rumen N balance and a negative MP balance produced significantly less milk than the positive control diet, but much more than currently predicted by either CPM Dairy v3.0 or NRC (2001). For cows fed to achieve a negative rumen N balance but a positive MP balance, a numerical but not significant decrease in milk yield was observed. There may have been a slight ruminal N deficiency in cows fed the diet predicted to be deficient in rumen available N (Diet T), as observed by a reduction in DMI. However, rumen NH_3 concentrations, PUN, and MUN values were not beyond the range observed previously in animals with no signs of ruminal N deficiency.

Plasma urea N, MUN, and ruminal NH_3 concentrations suggest that cows fed Diets P and N had higher ruminally available N than Diet T, as predicted during formulation. However, the magnitude of rumen N balance appears to be less defined as shown by comparison of ruminal NH_3 concentrations to predicted ruminal N balances among diets. No differences were observed in VFA concentrations, suggesting similar microbial fermentation.

Milk fat depression was observed across all diets, and was partially caused by the presence of monensin. Removal of monensin resulting in a 30% increase in milk fat. It was possible that the high concentration of corn based products exacerbated the MFD. Interpretation of production results is more difficult due to this effect, but the 3.5% FCM yield was lower in the two low CP diets with numerical differences in fat

yield among the three diets. Neither BW, BCS, nor reproductive parameters were affected by treatment.

Cows consuming Diet T had the highest N efficiency of use for milk, with cows consuming Diet P having the lowest N efficiency. This was reflected in MUN and PUN values and in the predicted proportion of N excreted in the urine, which was only approximately 150 g N/d for the two low CP diets, compared to 200 g N/d for the control CP diet. Feeding of these diets versus those with typical CP content (> 16% CP) has potential to result in less N excretion to the environment.

Future research is required to quantify the amounts of N that are not currently accounted for either at all or very accurately by current ration formulating software for dairy cattle at high levels of production. Such sources include recycled urea N, endogenous secretions, and intra-ruminal microbial turnover. Specifically, the amount of urea N recycled back into the GIT, along with the factors that influence it, should be determined more accurately for cows under a variety of production scenarios. More accurate predictions of endogenous protein flow will aid in predicting both ruminal peptide and MP supply. An inclusion of microbial turnover will better represent actual microbial growth, along with true rumen N availability. In addition, modifications in protein pools and rates of degradation may allow for improved prediction accuracy. All of these factors may have supplied enough N not currently predicted in Diets N and T to account for the lack of production response to low CP supply. If these mechanisms could be elucidated more quantitatively for dairy cattle in typical production scenarios, it would allow them to be fed at CP levels more closely aligned with requirements, resulting in less N excretion to the environment.