

DETERMINATION OF FEED UNAVAILABLE NITROGEN TO INCREASE PRODUCTIVE
EFFICIENCY IN HIGH PRODUCING DAIRY CATTLE

A Thesis

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ABSTRACT

The high cost of protein feeds and the growing concern for the environment has motivated dairy producers and nutritionists to focus their attention on increasing nitrogen (N) use efficiency in dairy farms. It is well recognized that reducing N content of cattle diets is the single most important factor to increase the efficiency of N use. However, to effectively feed lower protein diets requires the nutritionist to know the availability of N in feeds in order to not negatively affect milk production. Nutrition models are an essential tool to allow feeding lower protein diets. However, these models require precise characterization of N in feeds. A new assay was developed that predicts N indigestibility (unavailable N, uN) in non-forage feeds using an *in vitro* approach. This approach is known as the *in vitro* N indigestibility assay (IVNIDA). The predictions of this assay have not been prospectively evaluated in lactating dairy cattle as a primary experimental objective. The objective of this study was to evaluate in high producing dairy cattle, both the outcome of the IVNIDA and the ability to utilize the prediction of the uN assay in the Cornell Net Carbohydrate and Protein System (CNCPS) to predict cattle performance. To evaluate the uN assay predictions, a replicated pen study was conducted to assess the effect of balancing diets for uN on the performance of high producing dairy cattle. One hundred and twenty-eight cattle that were greater than 60 days in milk (DIM) at the beginning of the experiment were distributed into 8 pens of 16 cows and pens were randomly allocated to the two dietary treatments. Cattle were fed one of two iso-nitrogenous, iso-caloric and iso-NDF treatment diets where the only difference was from the inclusion of two different blood meals (BM) used in each diet. The uN content of the two BM was 9% and 34% as predicted by the assay, whereas with acid detergent insoluble nitrogen, no difference in indigestibility was expected. The inclusion of the BM was done on an iso-nitrogenous basis and

the formulated predicted difference in uN was 39 g/d or 5.8% of actual N intake, thus that represented the difference in available N between the two treatments. There was no effect of uN on dry matter intake (DMI) or N intake and averaged 27.3 kg/d and 668 g/d for both treatments, respectively. However, milk yield and energy corrected milk (ECM) were 1.6 and 1.9 kg/d higher for the cows fed the LOW uN diet ($P < 0.01$). Higher uN was also associated with lower milk protein yield ($P < 0.03$), lower milk fat yield ($P < 0.01$) and lower milk urea nitrogen ($P < 0.01$).

The result of the experiment indicated that IVNIDA predictions were consistent with cattle responses. An evaluation using the uN values in place of ADIN in the structure of the CNCPS demonstrated that MP allowable milk using the uN values from the uN assay increased the accuracy of the prediction and enabled the model to predict the first limiting nutrient when MP was first limiting. An economic analysis of digestibility indicated that even under the worst case scenario of high market price for blood meal and low milk price, utilizing a more digestible blood was associated with a beneficial economic impact for the dairy farm.

BIOGRAPHICAL SKETCH

Marcelo Gutierrez-Botero grew up in Manizales, Colombia. He attended to Granadino School from kinder garden to 12th grade. Throughout school he spend most of his free time at his family farm where he developed his passion for animals, specially horses and cows. Following his father example and his passion for animals he attended to Universidad de Caldas where he completed the degree of Medico Veterinario Zootecnista. He was offered to come to the United States for his internship at two veterinary clinics in the state of New York, where he met multiple Cornell alumni who cultivated his interest of pursuing his graduate degree at this institution. During one of his visits to campus he met Dr. Mike Van Amburgh, who encouraged him to apply to the University and work in his lab. Marcelo applied to Cornell and went home to defend his thesis, graduated from Veterinary School and worked at his family business or six months before moving to Ithaca, NY in August 2012 to begin his Masters.

A mi familia y amigos por su incondicionalidad.

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CHAPTER 1

LITERATURE REVIEW

1. Nitrogen Impact on the Environment

1.1. Introduction

Technological developments have significantly improved the quality of human life during the last 50 years. However, many of these new technologies have had a negative impact on the environment, thus contributing to climate change (Galloway, 1998). Most of these technologies were measured strictly in terms of their short and medium-term benefits, but the long-term effects were still not evident at that moment (Erisman et al., 2011). During the last decades, great concerns have arisen due to the long-term detrimental impacts of many current common practices on the environment and on human health (Rootes, 2003).

Agriculture is closely related to food production and the environment and thus, has been object of large attention as one of the sectors that plays a critical role in climate change (Bodelier and Steenbergh, 2014). In retrospective, significant advances have been achieved in this field to satisfy human demand for food. The growing demand for food and the fixed tillable land generated the need to maximize production per unit of land (Erisman et al., 2011). There are several key players in achieving that goal: management, plant and animal genetics, the application of synthetic fertilizers, balanced nutrition, etc.

The development of the Haber-Bosch process in the early 1900's enabled the industrial production of synthetic nitrogen (N) fertilizers. This practice produces NH_3 from atmospheric N_2 and H_2 in the presence of iron at high pressure and high temperature (Erisman et al., 2008). This process was originally developed by the German chemist Fritz Haber, to produce ammonia

for fertilizers and explosives. Ammonia used for explosives had a remarkable impact mainly during World War I and World War II, but the impact of synthetic fertilizers still prevails. During the green revolution, fertilizers had a positive impact due to the increase in food production (Erisman et al., 2007). However during the last few decades, there are growing concerns on the sustainability of the environment under the current highly N-dependent agricultural practices (Galloway, 1998).

1.2. Nitrogen in the Environment

Nitrogen is an essential nutrient for all plants and animals; it is required for the biosynthesis of protein, amino acids and essential nucleic components of the plant and animal cells. Even though N is the 7th most abundant element on earth, only 2% is found in the reactive form (**Nr**) (Galloway, 1998). Nr is found in nature in both organic and inorganic forms. Organic forms are bound to C, O or H, constituting proteins and urea, and inorganic forms includes ammonia (NH_3^-), ammonium (NH_4^+), amines and oxidized forms like nitrogen oxides (NO_x) (Sutton et al., 2011). The un-reactive form, di-nitrogen (N_2), constitutes 78% of the gases in the atmosphere (Erisman et al., 2008). In nature, N_2 is transformed to Nr by biological N fixation (**BNF**) and lightning. Nitrogen fixation is a microbe facilitated process that takes place in O_2 -free environments and directly fixes the N in the soil where it can be taken up by plants. It accounts for the fixation of 90-130 Tg (1 Tg = 1 million tons) / year on terrestrial environments, while 3-5 Tg N are fixed by lightning worldwide every year (Galloway, 1998). However, a significant portion of the fixed N is denitrified back to the atmosphere.

In nature, nitrification and de-nitrification are the processes responsible for maintaining N equilibrium. Nitrification describes the oxidation process by which ammonia is converted to nitrates (NH_3^-); and de-nitrification refers to the microbe-facilitated process responsible for the

reduction of NH_3^- and/or NH_2^- to N oxide forms. The latter can be further reduced to form di-nitrogen, N_2 (Hiscock et al., 1991). Today, with the high application rates of anthropogenic N adding up to the total amount of N being nitrified, de-nitrification is insufficient to prevent the accumulation of nitrites and nitrates in the environment (Oenema et al., 2007). This imbalance triggers the N cascade described by Galloway et al. (2003), resulting in the alteration of the greenhouse balance, the eutrophication of non-agricultural oligotrophic terrestrial and marine environments, soil acidification, among other devastating effects.

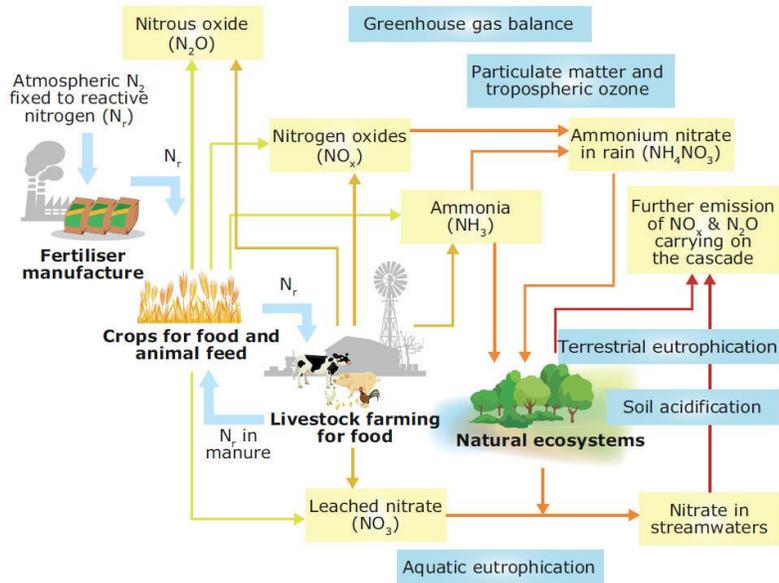


Figure 1.1. Nitrogen cascade as depicted by Sutton et al (2005).

1.3. Nitrogen in Agriculture

Agriculture contributes to approximately 80% of the production of N_r followed by industry (21%) and BNF (8%) (Sutton et al., 2011). The United States Environmental Protection Agency (EPA) reported that food production was the main driver of N use in this country, followed by combustion, housing and transportation, accounting for 58, 19, 12 and 12 kg N / capita, respectively (Leach et al., 2012). Erisman et al. (2005) reported that for every 100 Tg of N that

are used in crops, dairy, and meat, worldwide, only 17 Tg of N were actually consumed by humans. This alludes to losses derived from inefficiencies in the processes of converting N into food. Therefore, improving the efficiency of the use of N in the agricultural sector could have a significant impact on the reduction of overall N required per unit of food produced.

1.4. Nitrogen in Dairy Farming

The main sources for N losses in dairy farms derive from synthetic fertilizer use and cattle manure (Jones et al., 2014). Under today's feeding management schemes, manure N represents the main source of N output in dairy farming accounting for up to 80% of the total N (Tamminga, 1992, Tamminga, 1996). This form of N is typically utilized as a plant fertilizer and accounts for 25-30% of the total N applied to the fields; however, considerable amounts of N are lost in the form of NH_3^- and NO_3^- before the plant can make use of it (Sutton et al., 2011). Storage and field application account for the major loss of Nr in the form of NH_3^- with 19 and 17% loss, respectively (Oenema et al., 2007). Reynolds (2006) reported that NH_3^- from the manure was the most significant form of Nr lost into the environment contributing to the N cascade.

Different approaches have been proposed to reduce the impact of dairy farming on the environment. Oenema et al. (2007) reported a 90-230 kg N /ha difference between the surplus of farms that implemented nutrient efficient systems and those that did not. Appropriate manure management and adjustment of plant fertilization levels based on yield and efficiency of use, were found to be the main drivers of N surplus reduction (Oenema et al., 2007). Moreover, alternatives are available to increase the efficiency of nutrient use and uptake by the animal for its subsequent transformation into animal produce (Tamminga, 1996).

From the nutritional stand point, different possibilities exist that can reduce the amount of protein fed to the cows without affecting the level of production (Reynolds, 2006). However, reducing protein content in the diet needs to be compensated by an increase in the quality and/or availability of N for the cow (Calsamiglia et al., 2010). The successful reductions of protein content in the diets will directly impact the amount of feed required, thus affecting the quantities of fertilizer needed for feed production. Furthermore, feeding cows less quantity but with an increase in available protein can reduce the amount of N in manure, decreasing the production of NH_3 and NO from that source (Tamminga, 2003).

The recent development of more precise quantification tools have been made to better determine the impact of N in the environment (Leach et al., 2012). Better estimates describing N cycling should enable policymakers, producers and consumers to better describe and understand the entire N dilemma. This quantification should allow the determination of efficiencies, areas of major impact, regulation evaluations, etc. that should lead to better decisions in this regard (Leach et al., 2012).

1.5. Conclusions

Limited arable land availability affected by a growing demand for biofuel production and a fast growing human population with increased standards of living, dictate the future for agriculture and its impact on the environment (Galloway, 1998). An increasing demand for food per person, and an increasing number of people translate in the need to produce more food per unit of area to satisfy the future demands. Estimations based on the factors mentioned above state that the demand for fertilizers will double in the next 50 years if it continues to be unregulated (Galloway, 1998).

2. Nitrogen Digestion and Metabolism

2.1. Introduction

Ruminants evolved to survive on low nutrient density feeds (Van Soest, 1994). Well-developed symbiosis with rumen microbes has enabled ruminants to use high-fiber feeds more efficiently than most other herbivores (Van Soest, 1994). Complex interactions between feed, host, and rumen microbes determine the type and quantity of the nutritional substrates that reach the small intestine of the host (Nagaraja et al., 1997). Better understanding of rumen function has been used to maximize microbial protein yield in the rumen and feed rumen protected nutrients that are specifically targeted for absorption in the small intestine of the host (Russell et al., 1992, Schwab, 1995, Schwab, 1996).

2.2. Rumen Nitrogen Metabolism and Feed Degradation

Nitrogen metabolism in the rumen is the product of the interaction between feed, rumen microbes, and the host. Rumen degradation of N compounds results from the processes of solubilization and degradation (Bach et al., 2005). The former is driven by chewing and rumination, which allows the N components to dissociate from the plant cell structure and be exposed to the rumen microorganisms and free enzymes that would then degrade it (Rius et al., 2012). Large N-containing molecules such as proteins are broken down to oligo-peptides, di-peptides and free amino acids (Wallace et al., 1997). Amino acids can then be deaminated and their carbon backbone can then be used for energy or transformed into microbial protein (Cheeke and Derenfeld, 2010).

Rumen degradation and microbial protein synthesis are responsible for the transformation of N that arrives at the cow's small intestine following ingestion (Bach et al., 2005). The degree to

which microbes will incorporate N sources into microbial protein is highly dependent on carbohydrate availability (Calsamiglia et al., 2010). When insufficient carbohydrates are available, more AA are deaminated and the carbon backbone is used as fuel for microbial protein synthesis, resulting in ammonia accumulation in the rumen (Russell, 2009). The excess ammonia is absorbed by the rumen wall and drained into the portal circulation.

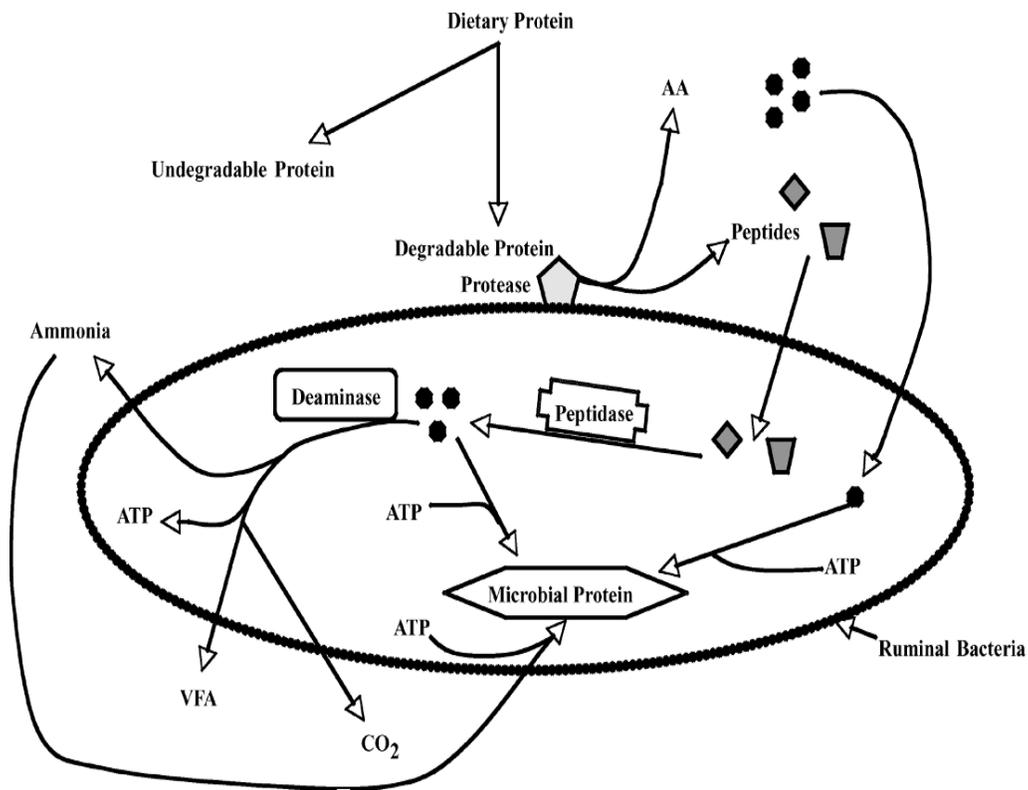


Figure 1.2. Schematic representation of protein degradation and fate of end products in the rumen (Bach et al., 2005).

The liver is responsible for detoxifying compounds that could potentially be harmful for the host (Reynolds, 2006). Ammonia is toxic to the central nervous system as it reduces the formation of ATP, leading to an energy deficit in the normal brain metabolism (Cheeke and Derenfeld, 2010). To remove the ammonia from the hepatic portal blood, the liver transforms it

into urea in the ornithine and the glutamine synthesis pathways. Ruminants have a two-way N recycling mechanism to support microbial protein synthesis when dietary N is scarce. Plasma urea N (**PUN**) can reach the rumen via the saliva or by passive diffusion through the rumen wall (Lapierre and Lobley, 2001).

Dietary N that is not incorporated in the metabolic processes of lactating dairy cattle will ultimately be excreted in feces, urine, or milk (Kauffman and St-Pierre, 2001). Fecal N is composed of undigested feed and bacterial N, enzymes, desquamated cells and other metabolic residues. Urinary N is highly variable and results from glomerular filtration in the kidneys (Figure 1.3) and is highly correlated with plasma urea N (Tamminga, 1996, Kauffman and St-Pierre, 2001).

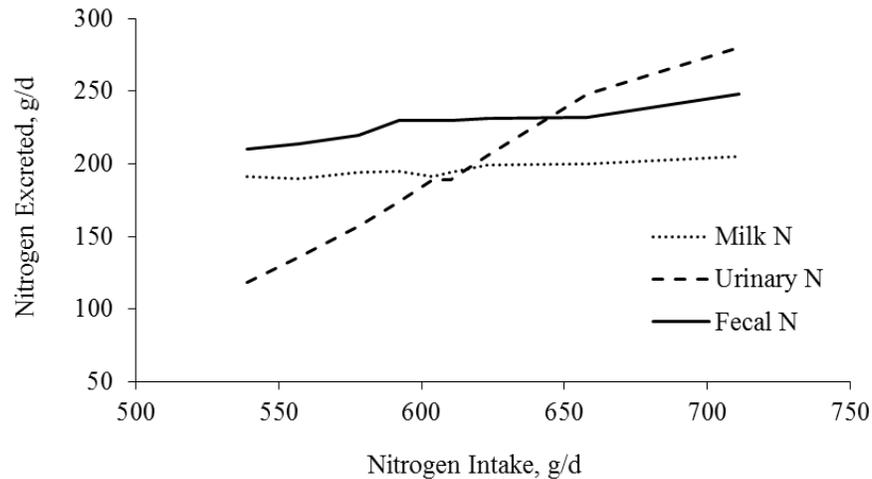


Figure 1.3. Nitrogen excretion as a function of nitrogen intake under conditions where energy is first limiting (Ipharraguerre and Clark, 2005).

Rumen bacteria produce proteases and peptidases which confers them the capacity to digest these nitrogenous molecules and transform them into microbial protein (Wallace et al., 1997). Dietary protein and non-protein N entering the rumen are the source of N for rumen microbes to grow and reproduce. No absolute requirements have been established for total microbial

population in the rumen. The cross feeding among bacteria has enabled them to meet their individual requirements (Russell, 2009).

Methods using heat and heat plus chemical agents, have been used to decrease the extent of rumen degradation (Faldet et al., 1991, Schwab, 1995). Using heat and moisture at the correct temperature increases rumen undegradable protein (**RUP**) content of the feed by denaturing proteins and creating protein-carbohydrate and protein-protein cross-links. Under-heating results in a lower content of RUP and over-heating will increase the RUP, but reduce its intestinal digestibility thus increasing its indigestible fraction (Van Soest, 1994). Treatments that include both heat and chemical agents consist of the addition of substances (ie. sugars) to the substrate, before applying heat and typically enhance the protein-carbohydrate cross-linking (Maillard reaction) (Van Soest and Mason, 1991). Metcalf et al. (1996) reported that increasing the inclusion of protected soybean meal in the diets resulted in increased milk production, milk protein yield and concentration in cattle fed 13.0, 15.1 and 20.4 % CP diets.

2.3. Nitrogen Post-Rumen Digestion and Absorption

Nitrogenous compounds in the small intestine are mainly microbial protein, rumen undegraded proteins and AA (Reynolds and Kristensen, 2008). Proteins are denatured so that amide bonds can be hydrolyzed into AA, which are then absorbed (Cheeke and Derenfeld, 2010).

Digestion of nitrogenous compounds is the preparation of these compounds for absorption in the small intestine (Wallace et al., 1997). This process starts in the abomasum with the secretion of pepsinogen by the chief cells of the gastric glands. Pepsinogen is a zymogen that is activated by HCl that is secreted from the parietal cells. Once activated it turns into the active enzyme,

pepsin. Pepsin is an endopeptidase which breaks down proteins into polypeptides by hydrolyzing peptide bonds.

Digestion occurs mainly in the small intestine. Here, proteins and polypeptides that are left after acid digestion in the abomasum, are split into free AA and small peptides that can then be absorbed by the animal. The secretion of trypsinogen, chymotrypsinogen and proelastase by the pancreas, serves a major role in the digestion of these compounds. These zymogens are activated in the small intestine and converted into endopeptidases, trypsin, chymotrypsin and elastases. Trypsin is specific to peptide bonds, chymotrypsin is specific to non-charged aromatic AA and elastin is a broad spectrum protease (Cheeke and Derenfeld, 2010).

Certain plants are known to contain pancreatic proteolytic enzyme inhibitors. Soybeans are a well-known source of trypsin inhibitors. These plants contain large protein molecules that have the ability to bind irreversibly to trypsin and chymotrypsin (Van Soest, 1994). Once these molecules bind to the enzyme, the enzyme loses its proteolytic capacity and gets excreted. Adequate heating inactivates trypsin inhibitors in soybean through the denaturation of the secondary structure of the protein molecule (Hung et al., 1984, Faldet et al., 1991).

The secretion of aminopeptidases and carboxypeptidases by cells in the brush border of the intestinal villi completes the process of protein digestion. The resultant free AA are readily absorbed. Amino acid absorption from the intestinal lumen to the enterocyte involves an active transport mechanism. These mechanisms are coupled to a sodium-phosphate pump to equilibrate the electric potential difference resultant from the process of absorption. Although small peptides are absorbed in smaller amounts, these are hydrolyzed into free AA in the erythrocytes.

Nitrogen requirements for the animal are calculated on the absorbable or metabolizable protein in the feed (NRC, 1989, 2001, Tylutki et al., 2008). Different methods to quantify

digestibility have been developed to determine the amount of feed N that is available for absorption by the cow.

3. Nitrogen Digestibility Determination

3.1. Introduction

Several approaches have been suggested to determine N digestibility in cattle feedstuffs. Goering et al. (1972) proposed using the portion of N bound to the indigestible fiber (ADIN) to determine the portion of N of the feed not available to the cow. Although this method was strongly correlated with intestinal digestibility in forages (Goering et al., 1972) it was less accurate determining the intestinal digestibility of non-forage ingredients like distiller grains (Weiss et al., 1989, Waters et al., 1992). The need for more precise methods to determine the digestibility of RUP in non-forage feeds led to the development of a series of techniques that have been able to provide a more physiological representation of the digestive physiology of the cow (Metcalf et al., 1996).

3.2. In Vitro Enzymatic Methods

In vitro techniques to quantify intestinal digestibility of protein have been developed to reduce the variability due to animal differences, reduce the cost of the analysis, and reduce the need of cannulated cows, which are unavailable in some countries (Stern et al., 1997). Most enzymatic *in vitro* techniques to determine protein intestinal digestibility follow a similar protocol of incubating samples at an ideal temperature using a single or a mix of enzymes (Akeson and Stahmann, 1964, Hsu et al., 1977). After completing the enzymatic reaction,

digestibility is determined based on the small peptides and free amino acids released. Techniques differentiate in the time of exposure and the enzymes or mix of enzymes utilized.

Single-enzyme methods have shown to have variable responses associated to the specificity of the enzyme utilized for individual peptide bonds (Mahadevan et al., 1987). The values for intestinal digestibility of single-enzyme methods tend to be more dependent on the number of bonds that are sensitive to the enzyme utilized rather than the intestinal digestibility per se (Mahadevan et al., 1987). Therefore, new physiological approaches for protein digestion should be developed to better represent the natural conditions present in the animal.

The method developed by Akeson and Stahmann (1964) simulated both the acid digestion that takes place in the abomasum and the intestinal digestion that takes place in the duodenum using pepsin and pancreatin, respectively. The results from this experiment on twelve food proteins were compared to a rat growth trial and a correlation of 0.99 was observed. However, the application of this technique in animal nutrition has been limited (Stern et al., 1997).

A method to quantify intestinal digestion based on changes in pH was developed by Hsu et al. (1977). This procedure utilized an enzyme mix containing pepsin, trypsin, and chymotrypsin in a solution at a pH of 8. The pH measurements after 10 minutes had a 0.9 correlation with *in vivo* estimates of intestinal digestion of protein in rats. The low cost and simplicity of this procedure make this technique potentially appealing for commercial labs. However, equations would need to be developed for each feed type which limits the commercial applicability of this technique. Furthermore, the application of this method to quantify intestinal digestion of protein can be affected by the buffer-intrinsic-capacity of feeds, which can be affected by rumen incubation (Hung et al., 1984).

3.3. *In Vivo Methods*

The *in vivo* mobile bag technique (**MBT**) was originally developed to determine digestibility in swine and was then adapted to measure post rumen digestion in ruminants (de Boer et al., 1987). This method pre-incubates the samples in the rumen, followed by an incubation in an HCl-pepsin solution, and final introduction into the duodenum of the cow. Samples can either be collected in the ileum or in feces. Bags are then washed to remove any endogenous and microbial protein. This last step theoretically enables the method to measure true digestibility, instead of apparent digestibility (Robinson et al., 1992).

The main sources of variation of the MBT are related to the porosity and size of the bags, ruminal pre-incubation, retention time, site of recovery, animal diet and bacterial contamination (Hvelplund, 1985, Voigt et al., 1985). Porosity of the bags range from 9 to 80 μm . Despite the wide range in pore size, protein digestion was affected to a greater extent by the surface area of the bags. For forages, bags with a smaller surface area show reduced protein digestion (Stern et al., 1997).

The effect of rumen pre-incubation has been variable among feeds. The pre-incubation step increases the intestinal digestibility on barley, oats, heat-treated canola, meat, bone meals and some forages (Volden and Harstad, 1995). However, Hvelplund et al. (1992) reported negligible effects of rumen pre-incubation when determining intestinal protein digestibility in soybean meal, peas, cottonseed cake, grass silage and whole-plant barely silage.

Different researchers have reported contradicting results for the effect of HCl pre-incubation in feed digestibility. Bruchem et al. (1985), Graham et al. (1985), Voigt et al. (1985) concluded that the effect of HCl was insignificant for the determination of intestinal protein digestibility. However, Finlayson and Armstrong (1986) reported that for heat and chemically protected

proteins the HCl pre-incubation was required to predict the intestinal digestibility of protein. Calsamiglia and Stern (1995) reported that pre-incubating samples in HCl increased the pancreatin digestion of blood meal, corn gluten meal, feathers meal, hydrolyzed feather meal, meat and bone meal and soybean meal.

Retention time of the bags in the intestine vary widely depending on the place of recovery (Norberg et al., 2007). Bags can be recovered either in the ileum or in feces. Although ileal recoveries are a more physiological representation of the protein that is absorbed by the cow in the small intestine, fecal recoveries are more practical and reduce the need for ileal cannulated cows (Paz et al., 2014). However, bags collected in the feces are exposed to microbial fermentation in the large intestine and may be prone to contamination with microbial protein present in the lower tract. Vanhatalo et al. (1995) reported that the apparent intestinal digestion of rapeseed and soybean meals was increased when bags were recovered from feces instead of from ileum. Nevertheless, meat and bone meal and grass silage were not affected by the site of recovery in the same study.

The MBT was highly correlated to *in vivo* intestinal digestibility ($r = 0.81$) as reported by Hvelplund (1985) and to rat growth trials ($r = 0.92$) as reported by Rooke (1985). However, the significant interaction found between the feed ingredient and the site of recovery of the bags make the validity of the data questionable (Calsamiglia and Stern, 1995).

3.4. The Three Step Procedure

A methodology to quantify intestinal digestion of proteins containing both *in vivo* and *in vitro* procedures was developed by Calsamiglia and Stern (1995). The technique used Dacron bags to suspend the sample in the rumen. The residue was then incubated in an HCl-pepsin solution for 1-hour. Samples were then neutralized by adding 1 N NaOH, and a pH=7.8 buffer containing

pancreatin. Samples were subsequently incubated for 24h and the undigested proteins were precipitated using TCA solution.

The ruminal pre-incubation, pepsin digestion and the pancreatin digestion steps were assessed to validate the technique. Rumen pre-incubation had no effect on the pepsin-pancreatin digestion for blood, soybean and corn gluten meals, but a decreased digestion was observed for fish, meat and bone, and hydrolyzed feather meals. The pepsin-digestion step enhanced the digestion of all the ingredients tested by 23 units on average (Calsamiglia and Stern, 1995). There was a 0.91 correlation between the pancreatin test and *in vivo* estimates. This procedure was adopted as the reference method for intestinal digestibility measurements by the NRC (2001)

Gargallo et al. (2006) presented a modified version of the three step procedure to determine intestinal digestibility of protein. This modified technique used an *in vitro* Daisy^{II} batch incubator (Ankom Technology, Macedon, NY) to reduce the cost and labor required and to eliminate the use of trichloroacetic acid due to its potential human and environmental hazards (Gargallo et al., 2006). The same authors reported a correlation (0.84) between the original and the modified three-step procedure using soybean meal that was heated at 170°C for 0, 0.5, 1, 2, 4, 6, and 8 h, suggesting that the modified method was a reliable procedure to determine intestinal digestibility in cattle.

Further refinements of the existing methods were focused on determining the amino acid profile of the RUP. Boucher et al. (2009) evaluated a precision fed cecectomized rooster bioassay to allow the recovery of a final residue that could be analyzed for individual amino acids. Ross et al. (2013) developed the *in vitro* N indigestibility assay, which also allowed for the quantification of amino acids in the RUP.

3.5. The In Vitro Nitrogen Indigestibility Assay

The *in vitro* protein indigestibility assay (IVNIDA) described by Ross et al. (2013) was developed to determine N indigestibility (uN) in cattle. This assay was designed for commercial use and to provide a more physiological estimate of protein digestibility in cattle than that provided by existing methods. The assay is a refinement of existing *in vivo* and *in vitro* methods to determine the portion of N that is unavailable to the cow. It simulates the rumen fermentation, gastric digestion and intestinal digestion; aiming to reflect the conditions of the gastrointestinal tract of the cow *in vitro*.

The uN assay has four major differences from existing methods that make it more physiological and commercially viable. First, it is a completely *in vitro* technique once the rumen fluid is collected from rumen-cannulated cows. Second, it replaces nylon bags with Erlenmeyer flasks to reduce lag time and sample loss of small indigested particles (Paz et al., 2014). Third, it uses a more physiological enzyme mix that provides more specific enzymatic activity and reduces the variation observed when using pancreatin (Ross et al., 2013). Last, it replaces trichloroacetic acid precipitation with filtration, thus increasing recovery of undigested feed particles (Ross et al., 2013). Glass microfiber filters utilized in this procedure have been reported to be chemically inert, resistant to heat and acid, and slightly hydrophobic (Raffrenato and Van Amburgh, 2011), thus reducing variability associated with filtering.

4. Conclusions and Objectives

Nitrogen is an essential nutrient that is required by both plant and animal cells for the synthesis of proteins, amino acids and other essential nucleic constituents. In nature, nitrification and de-nitrification maintain the reactive and un-reactive N in equilibrium. Dairy farms through

livestock production represent a significant contribution to the N emission. Yet, different management strategies that allow an increase in the efficiency of N used by the cow are available. Efforts to reduce N emissions without affecting levels of production have been studied and positively correlated to reducing the N content of the diets (Tamminga, 1992). However, reduction of the N content of the diets requires utilizing sources of N that are available or digestible to the cow.

To better understand N availability in diets, precise methods to quantify N unavailability in feedstuffs are required. To provide a more physiological estimation of N unavailability and allow the determination of the digestibility of individual amino acids, our lab has developed an *in vitro* N indigestibility assay. The main purpose of this assay is to provide nutritional models like the Cornell Net Protein and Carbohydrate System (CNCPS) more precise information that allows for reduced N diets without decreasing milk production.

The objectives of this thesis are to assess the results of the IVNIDA and to evaluate the ability of the CNCPS to predict cattle performance using the uN obtained from the IVNIDA.

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CHAPTER 2

FORMULATING DIETS FOR INTESTINAL NITROGEN INDIGESTIBILITY IN HIGH PRODUCING DAIRY CATTLE AND APPLICATION WITHIN THE STRUCTURE OF THE CNCPS

1. Abstract

The high cost of protein feeds and the growing concern for the environment has motivated dairy producers and nutritionists to focus their attention on increasing nitrogen (N) use efficiency in dairy farms. It is well recognized that reducing N content of cattle diets is the single most important factor to increase the efficiency of N use. However, to effectively feed lower protein diets requires the nutritionist to know the availability of N in feeds in order to not negatively affect milk production. A new assay to estimate intestinal N indigestibility (unavailable N, uN) was developed and it was important to evaluate the predictions of this assay in lactating cattle to ensure that the assay could reasonably estimate the uN in feeds at a resolution the cattle were sensitive to. To evaluate the assay's predictions, a replicated pen study was conducted to evaluate the effect of uN on the performance of high producing dairy cattle. One hundred and twenty-eight cattle that were 97 to 147 days in milk at the beginning of the experiment were distributed into 8 pens of 16 cows and pens were randomly allocated to the two dietary treatments. Cattle were fed one of two iso-nitrogenous, iso-caloric and iso-NDF treatment diets where the only difference was from the inclusion of two different blood meals (BM) used in each diet. The uN content of the two BM was 9% and 34% unavailable as predicted by the assay, whereas as measured by analysis of ADIN, no difference in indigestibility was expected. The inclusion of the BM was on an iso-nitrogenous basis and the predicted difference in uN was 39 g/d or 5.8% of actual N intake, thus that represented the difference in available N between the

two treatments. There was no effect of uN on dry matter intake or N intake and averaged 27.3 kg/d and 668 g/d for both treatments, respectively. However, milk yield and energy corrected milk were 1.6 and 1.9 kg/d higher for the cows fed the LOW uN diet ($P < 0.01$). Higher uN was also associated with lower milk protein yield ($P < 0.03$), lower milk fat yield ($P < 0.01$) and lower milk urea nitrogen ($P < 0.01$). An evaluation using the uN values in place of ADIN in the structure of the Cornell Net Carbohydrate and Protein System demonstrated that MP allowable milk using the uN values in place of ADIN increased the accuracy of the prediction and enabled the model to predict the first limiting nutrient provided all of the other feed, cattle and management characteristics were also defined.

Key words: intestinal digestibility, unavailable nitrogen, feed chemistry, milk yield, nitrogen efficiency.

2. Introduction

Current cattle diet formulation models rely on library estimates of intestinal indigestibility of proteins and carbohydrates to predict metabolizable energy (**ME**) and metabolizable protein (**MP**) supply (Tylutki et al., 2008). As models become more accurate and precise in the prediction of nutrient supply and nutrient balance, there is a greater need to evaluate and be able to adapt the inputs currently implemented as static library values. Although crude protein (**CP**) is not a functional dietary nutrient for cattle (Ipharraguerre and Clark, 2005), many dairy cattle diets are still formulated on this metric, creating confusion due to inadequate information provided by the measure, especially with regard to MP supply and amino acid availability. As diets are formulated closer to the MP requirements of cattle and subsequently lower in CP, accurate estimates of intestinal digestibility (**ID**) of protein and amino acids are increasingly important to ensure an adequate supply of those nutrients. Use of outdated feed library values

for all feeding conditions can lead to under- and over-estimations of MP and amino acid supply, resulting in variation from expected or predicted production.

Since the inception of the Cornell Net Carbohydrate and Protein System (**CNCPS**) the detergent method of fractionation has been applied to both the carbohydrate and protein components of feeds (Sniffen et al., 1992, Tylutki et al., 2008). More recent work suggests this approach might not be appropriate to accurately characterize how protein is partitioned and digested in the rumen and post-ruminally, especially for feeds not containing NDF. Several approaches have been developed to predict the intestinal digestibility of protein in feeds and are a departure from the detergent method of feed chemical composition (Calsamiglia and Stern, 1995, Boucher et al., 2009, Ross et al., 2013). Nearly all of the published data from these methods provide comparisons to other feeds, and not to cattle performance (Gargallo et al., 2006; Boucher et al. 2009). Very few published studies exist where the estimates of the methods are evaluated in a prospective feeding study, especially where cattle are fed N intakes at a level that makes them sensitive to the differences in digestibility (Noftsker and St-Pierre, 2003).

Further, blood meal (BM) is a byproduct derived from meat production that is used as a protein supplement in cattle. After collected from the animals in a liquid form, the blood is dried to facilitate transportation and handling. The application of high temperatures for extended periods of time during the drying process can result in reduced BM digestibility when fed to cattle. Blood meal is traded in the commodity market and its value is based on the price of soybean meal (**SBM**) without taking into account its N availability. Because it contains no NDF or ADF, use of those assays to evaluate N availability within the rumen or post-ruminally does not provide adequate information for diet formulation.

Thus, the objective of this study was to test 1) the concept of unavailable nitrogen (**uN**) in lactating dairy cattle using the estimates provided by the assay of Ross et al. (2013) and 2) the ability of the CNCPS to predict MP allowable milk using the uN predicted from the assay of two different blood meals in place of acid detergent insoluble N (**ADIN**).

3. Material and Methods

3.1. Animals, Treatments and Experimental Design

All procedures involving the use of animals were approved by the Cornell University Animal Care and Use Committee. Ninety-six multiparous cattle (726 ± 14 kg BW; 147 ± 64 DIM; 45 ± 48 DCC) and thirty-two primiparous cattle (607 ± 30 kg BW; 97 ± 20 DIM; 21 ± 28 DCC) were distributed by days in milk (**DIM**) and body weight (**BW**) into 8 pens of 16 cows (12 multiparous and 4 primiparous). Pens were stratified into four levels of milk production, and each stratum randomly allocated to treatments.

Diets were formulated with the CNCPS v6.1 (Tylutki et al., 2008, Van Amburgh et al., 2010) using the measured wet chemical composition of the ingredients used in the treatment diets (Table 2.1). Ingredients for the experimental diets were the same for each treatment except for the two different BM products. The two BM differed by their overall N content and the uN analysis and contained 9% and 34% uN for the low uN (**LOW uN**) and high uN (**HIGH uN**) diets, respectively. The two dietary treatments were formulated by inclusion of the BM on an iso-N basis and to maintain iso-nitrogenous diets, due to the differences in the N content of the two BM, the HIGH uN diet had a 0.3% greater inclusion of BM (Table 2.2). This resulted in minor adjustments in the inclusion of sodium bicarbonate, soy hulls and wheat midds, which were reduced by 0.1% of dry matter (**DM**) each in the HIGH uN treatment.

To adjust for changes in MP supply in both treatments due to the changes in milk yield related to the stage of lactation and subsequent MP requirements, the protein content of both diets was reduced during the 5th week of study. As the cows progressed into later lactation a re-evaluation of both treatment diets and cattle data was conducted using CNCPS to evaluate the predictions between ME and MP allowable milk and MP supply in an effort to ensure the cattle remained sensitive to the dietary differences in uN. The evaluation determined the cattle were being overfed MP relative to MP requirements, thus the canola meal was reduced in both diets by 50% to be consistent with the ME allowable milk and to maintain the MP and N supply at a level the cattle should remain sensitive to the treatment differences in N availability created by the inclusion of the two different BM. This is also why a longitudinal study design was employed and not a Latin square or cross-over. In a Latin square design, there could be no adjustment to the N intake, thus in subsequent periods, the possible treatment effect would be lost due to overfeeding N as the cattle progressed into later lactation and the MP requirements were decreasing.

The lactation trial consisted of a 2-wk adaptation period, a 1-wk covariate period and a 9-wk experimental period, between March 30 and June 14, 2014 at Cornell University Ruminant Center (Harford, NY). All cows were fed the LOW uN diet during adaptation and covariate periods. Cows were housed in pens in a four row barn design with one sand bed and more than one headlock per cow and free access to water. All cows received rBST (Posilac, Elanco Animal Health, Indianapolis, IN) on a 14-d schedule throughout the length of the trial on the same schedule.

Table 2.1. Nutrient composition of ingredients in HIGH and LOW unavailable nitrogen (uN) diets.

Item ¹	Ingredient ²								
	Alfalfa silage	Corn silage	BM low uN	BM high uN	Bakery by- product	Canola meal	Corn meal	Soy hulls	Wheat midds
DM, % as fed	36.2	41.1	92.8	92.1	91.4	89.1	86.3	87.1	88.3
CP, % DM	22.4	7.8	94.0	91.2	13.1	46.5	7.9	11.5	18.6
SP, % CP	58.8	49.2	28.4	5.55	24.7	21.9	16.6	27.0	43.3
aNDF, % DM	43.5	38.4	---	---	10.6	29.9	7.8	71.0	39.2
ADF, % DM	36.5	24.8	---	---	6.63	20.3	2.8	49.8	12.2
NDICP, % CP	12.3	14.5	---	---	11.5	12.9	6.27	33.4	16.5
ADICP, % CP	8.6	11.6	---	---	7.07	9.80	3.87	9.30	4.33
LIGNIN, % DM	8.06	2.55	1.24	1.69	2.04	10.8	1.00	2.37	3.61
uN, % total N	26.9	21.2	8.99	33.8	21.2	12.5	17.2	17.3	0.72
EE, % DM	3.89	3.39	0.18	2.19	5.28	3.71	3.60	1.26	3.88
Sugar, % DM	2.70	1.40	1.05	1.10	18.3	11.0	3.77	2.33	6.60
Starch, % DM	0.87	34.1	0.27	0.25	43.3	0.60	74.9	0.17	23.3
Ash, % DM	10.6	3.54	3.41	3.86	4.20	8.45	1.67	5.92	6.01
Ca, % DM	1.43	0.18	0.02	0.46	0.18	1.08	0.01	0.67	0.12
P, % DM	0.42	0.26	0.39	0.19	0.43	1.18	0.30	0.11	1.20
30 hr NDF kd, ³	4.60	3.62	----	----	----	---	---	---	---

¹ DM: dry matter; CP: crude protein; aNDF: neutral detergent fiber analyzed using a heat stable alpha-amylase and sodium sulfite; ADF: acid detergent fiber; uN: unavailable N; ME: metabolizable energy; EE: ether extract.

² BM: blood meal.

³ (% / hr)

Table 2.2. The formulated diet composition of two diets with LOW and HIGH unavailable nitrogen (uN).

Ingredient ¹ , % DM	Treatment	
	LOW uN	HIGH uN
Alfalfa silage	11.5	11.5
BMR corn silage	49.3	49.3
Bakery byproduct	1.8	1.8
BM low uN	3.7	---
BM high uN	---	4.0
Canola meal	3.0	3.0
Corn grain	16.1	16.1
Energy Booster 100	1.8	1.8
Molasses	1.8	1.8
Smartamine M	0.1	0.1
Sodium bicarbonate	0.6	0.5
Soybean hulls	4.6	4.5
Urea	0.2	0.2
Wheat midds	4.6	4.5
Min/vit mix ²	1.0	1.0
<i>Chemical composition</i>		
DM, % as fed	49.7	50.9
CP, % DM	15.2	15.2
NDF, % DM	31.9	32.3
ADF, % DM	21.3	20.5
EE, % DM	4.26	3.91
Starch, % DM	30.4	31.2
Sugar, % DM	3.60	3.27
Ca, % DM	0.65	0.60
P, % DM	0.43	0.43
ME*, mcal/kg DM	2.61	2.60
Met*, % MP	2.26	2.28
Lys*, % MP	7.36	7.26
Lys:Met*, % MP	3.25	3.18

¹ BM: blood meal; DM: dry matter; CP: crude protein; aNDF: neutral detergent fiber analyzed using a heat stable alpha-amylase and sodium sulfite; ADF: acid detergent fiber; NEI: net energy for lactation; EE: ether extract.

² Mineral/vitamin mix contained 243 g Ca⁺; 70 g P; 8.6 g Mg; 8.1 g K; 14.6 g S; 47 g Na⁺; 72 g Cl⁻; 50 ppm I; 0.98 ppm Co; 507 ppm Cu; 4115 ppm Fe; 1331 ppm Zn; 485 ppm Mn; 25ppm Se; 342 KUI vitamin A; 70 KUI vitamin D; 2,512 KUI vitamin E and 1.8g monensin; per kg of dry matter.

* calculated with the CNCPSv6.1 (Tylutki et al, 2008; Van Amburgh et al., 2010)

3.2. Sampling Procedure

Cows were fed a total mixed ration (**TMR**) once per day at 0600 h targeting 5-8% refusals. A single batch of the diet/day was mixed and delivered to the four pens assigned to each treatment. Feed offered and refused was recorded using Feed Watch (Valley Agricultural Software, Tulare, CA). Weekly forage, TMR and pen refusals samples were collected from the bunks at feeding and cleaning time, respectively. Two subsamples were obtained, one used for DM and further N determination and one stored at -20°C. On farm DM was determined twice weekly using a moisture tester (Koster Crop Tester Inc., Brunswick, OH) and the Feedwatch software was adjusted accordingly.

Cows were milked three times per day at 0600 h, 1400 h and 2200 h and data from all milkings were recorded using the Alpro herd management system (DeLaval International AB, SG). Individual milk samples were collected weekly during three consecutive milkings, and preserved with 2-bromo-2-nitropane-1, 3-diol at 4°C until analyzed. Milk yield was expressed as actual milk yield and 3.5% energy corrected milk (ECM) according to the equation of Tyrrell and Reid (1965): $ECM (kg) = (12.82 * kg \text{ fat}) + (7.13 * kg \text{ true protein}) + (0.0323 * kg \text{ milk})$.

Cows were weighed once per week using a platform scale XR3000 (Trutest, NZ) after the morning milking. Further, body condition score (**BCS**) on a scale of 1 to 5 (Wildman et al., 1982) was observed every 2-wk by the same two evaluators. An average of the two evaluators was used as the mean BCS for each week.

3.3. Sample Preparation and Analysis

Milk samples were analyzed by mid-infrared methods (DairyOne, Ithaca, NY) for fat, true protein, lactose, total solids, MUN (Foss Milkoscan FT+, Foss In., Eden Prairie, MN; AOAC, 1990) and somatic cell count (SCC; Fossomatic FC, Foss Inc., Eden Prairie, MN; AOAC 1990).

Forages and TMR offered and refused were dried in triplicate in a forced air oven for 48 h at 55°C and ground using a Wiley mill (Arthur H. Thomas, Philadelphia, PA) with a 2-mm screen. Weekly samples were pooled by month and analyzed for dry matter at 105°C for DM and ash (AOAC, 1990), NDF and ADF using heat stable α -amylase (Van Soest et al., 1991), total N, NDIN and ADIN by combustion assay (Leco Instruments Inc; AOAC, 2000), fat (AOAC, 2006), starch (Hall, 2008) and sugar (Dubois et al., 1956) (Cumberland Valley Analytical Services, Hagerstown, MD).

The uN in the two BM was determined according to the *in vitro* N indigestibility assay described by Ross et al. (2013). Briefly, 0.5 g of sample was placed into a 125 ml Erlenmeyer flask. Forty (40) ml of rumen buffer (Goering and Van Soest, 1970) and 10 ml of rumen fluid were added to each flask. Flasks were incubated in a water bath at 39°C under continuous CO₂. After 16 h, samples were acidified with 2 ml 3M HCl to stop the microbial fermentation. Samples were then incubated on a shaking bath after adding 2 ml of pepsin and adequate HCl to achieve a pH of 2. After one hour of simulated gastric digestion, the samples were neutralized with 2 ml of 2M NaOH to stop the pepsin reaction. An enzyme mix containing trypsin, chymotrypsin, lipase and amylase was added to the flask and incubated in the shaking bath at 39°C. After 24 h, the samples were filtered on 90 mm, 1.5 μ m glass filter (Whatman 934-AH, GE Healthcare Bio-Sciences Corp., Piscataway, NY 08855) and rinsed with hot distilled water. The N content of the residue was determined by Kjeldahl and expressed as a percent of the total N in the sample.

3.4. Calculating uN within the Structure of The CNCPS

This experiment allowed us to compare the effect of two different feed chemistry approaches to estimate uN values for BM. One method used a combination of the neutral detergent insoluble

N (**NDIN**) and ADIN values, and the second was the value determined by the uN assay. The detergent method partitions the N linked to cell wall and the ADIN, or C pool within the framework of CNCPS, and is considered indigestible and therefore has a no ruminal degradation rate and zero intestinal digestibility. The NDIN has some ruminal digestibility based on the integration of the rates of digestion and passage and after calculating ruminal escape, the intestinal digestibility of the escape protein is 80% (NRC, 1989, Sniffen et al., 1992). To apply the uN values determined by the *in vitro* N indigestibility assay required the following adjustments to the CNCPS feed inputs. By definition and analysis BM contains no aNDFom (Mertens, 2002), and therefore no NDIN. With this approach, all of the protein in BM is in the soluble true (**A2**) and insoluble true (**B1**) and indigestible (**C**) fractions. For the two BM, the uN values from Ross et al. (2013), were substituted in place of the ADIN. Zero degradation rate for that pool was maintained since the assay determined that portion is 100% unavailable. The remaining N fractions were assigned a digestibility of 100 %.

3.5. Statistical Analysis

Data were analyzed using a mixed effects model (JMPv.11 SAS Institute, Inc., Cary, NC):

$$Y_{ijkl} = T_i + W_j + TW_{ij} + B_l + ck(P) + E_{ijkl}$$

where Y_{ijkl} is the dependent, continuous variable; T_i is the fixed effect of the i th treatment ($i=1, 2$); W_j is the fixed effect of the j th week ($j=1, \dots, 9$); TW_{ij} is the fixed effect of the interaction between the i th treatment and the j th week; B_l is the covariate measurement for the l th ($l=1, \dots, 128$) cow or the l th pen ($l=1, \dots, 8$), depending on the variable tested; ck is the random effect of the k th cow nested within pen or k th pen, depending on the variable tested; and E_{ijkl} is the residual error. The statistical unit for milk yield, milk components, BW and BCS was the random

variable cow nested within pen, whereas for dry matter intake (**DMI**) and N intake was the random variable pen.

The balanced design (equal number of cows per pen and equal number of pens per treatment) allowed for the use of animal as the error term for the analysis of milk, BW and BCS; and permitted the exclusion of random variable pen from the model, without overestimating the error degrees of freedom of the model (St-Pierre, 2007). Regression analysis was used to calculate weight gain for individual cows accounting for the need to include week in the statistical model for that variable. Overall treatment differences were evaluated using least square means. Significance was declared at P-values < 0.05.

3.6. Economic Analysis of Digestibility

An economic analysis around the concept of digestibility/indigestibility was performed. The analysis was presented in two scenarios. In both cases it was assumed that at least two blood meals with different digestibility were available for the same market price (Stucker (2014), personal communication). The first analyzed the impact of the digestibility of BM on feed cost; and the second evaluated the potential opportunity cost of utilizing a BM with unknown digestibility. Both scenarios were based on the results of this experiment; however, the analysis was not limited to the experimental conditions. In both cases, a sensitivity analysis was performed evaluating changes in feed price and milk price, respectively.

The first scenario was a comparison between using two types of BM with different digestibility to provide 75 g of digestible N in a diet, the amount of digestible N expected by the inclusion of the BM to meet the formulated MP requirements. These values correspond to feeding approximately 0.5 kg / cow / d to represent typical field conditions. It was assumed that both BM had the same N content (16%) and the same market value (\$1480/ton; average for 2014

calculated from UW-Madison FeedVal 2012:

http://dairymgt.info/tools/feedval_12_v2/index.php. The sensitivity analysis was conducted under three scenarios where the market value of BM was 2, 3 and 4 times the average market value of SBM during 2014. Results from the sensitivity analysis were presented as the opportunity cost per 500 lactating dairy cattle per month for every percentage point in digestibility different between the two hypothetical BM.

The second scenario evaluated the potential opportunity cost derived from feeding a BM with unknown digestibility assuming the actual digestibility was lower than expected. The analysis was based on the results observed in the experiment assuming a linear response to increase N availability. Average milk price was calculated from actual monthly prices paid to the CURC Dairy during 2014. The sensitivity analysis simulated three hypothetical scenarios where price of milk was high (\$25), intermediate (\$18), and low (\$11) per hundred pounds of milk. Milk prices used were based on component pricing, and average prices for CURC during 2014. The price ratio between milk components was unchanged in the three scenarios. Results from the sensitivity analysis were presented as the increase in revenue per 500 lactating dairy cattle per month per percentage point in digestibility.

4. Results

4.1. Animal Performance

Dry matter intake (on average 27.3 ± 1.7 kg/d) and N intake (on average 668 ± 47 g/d) were not different between treatments (Table 2.3). Observed N intakes by treatment and by week of experiment are presented in Figure 2.1. At the levels of intake observed in this experiment and after accounting for the rates of inclusion, N content and inclusion rates of the BM, the cattle fed

the HIGH uN diet consumed 38 g more uN than the LOW uN diet and this represented the difference in N availability between the treatments.

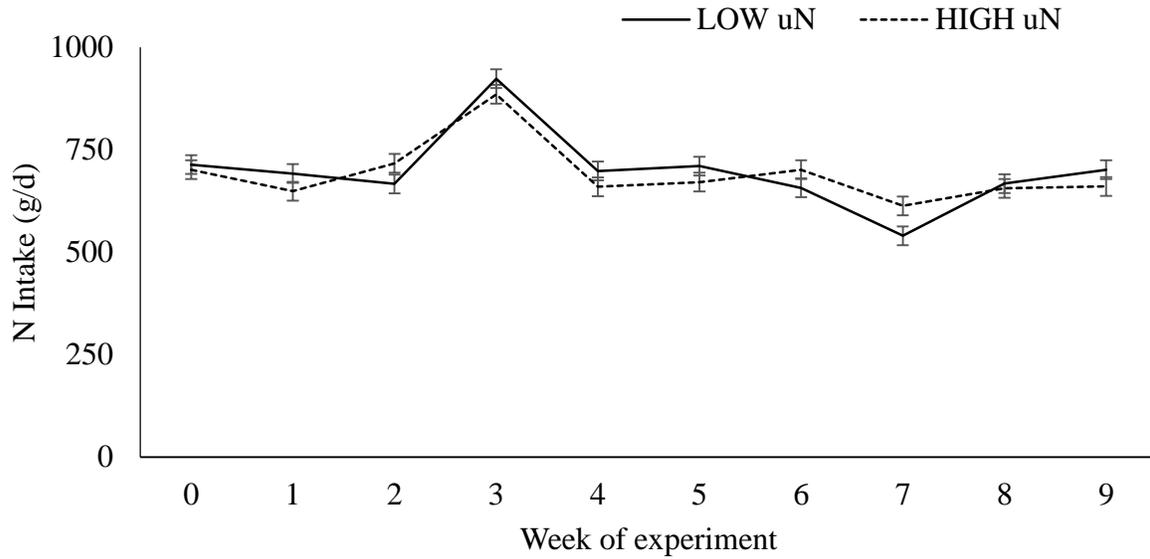


Figure 2.1. Least square means nitrogen intake for cattle fed LOW and HIGH unavailable nitrogen (uN) diets.

Table 2.3. Effect of N availability on intake, milk production, milk composition and body weight gain of dairy cows fed LOW and HIGH unavailable nitrogen (uN) diets.

Item ¹	Treatment ²		SEM	P-value
	LOW	HIGH		
DMI, kg	27.4	27.1	0.61	0.75
N intake, kg	671.1	664.4	14.8	0.77
<i>Milk production</i>				
Milk, kg	42.0	40.4	0.31	<0.01
ECM, kg	41.9	40.0	0.32	<0.01
Fat, kg	1.51	1.42	0.02	<0.01
Protein, kg	1.26	1.23	0.01	0.03
<i>Milk composition</i>				
Fat, %	3.65	3.55	0.03	<0.03
Protein, %	3.03	3.06	0.02	0.20
Lactose, %	4.90	4.86	0.02	0.18
MUN, mg/dl	9.4	8.0	0.18	<0.01
SCC, log(1000/ml)	3.9	4.0	0.05	0.13
<i>BW and BCS</i>				
BWinitial, kg	684.1	692.1	10.1	0.58
BWchange kg	34.7	29.7	2.25	0.12
BCSchange	0.20	0.16	0.03	0.29
<i>Efficiency</i>				
Gross feed efficiency ³	1.56	1.50	0.03	0.34
Milk N efficiency ⁴	30.0	29.7	0.70	0.76

¹ DMI: dry matter intake, ECM: energy corrected milk yield (Tyrrell and Reid, 1965), MUN: milk urea nitrogen, SCC: somatic cell count.

² LOW: low uN diet, HIGH: high uN diet.

³ calculated as kg milk / kg DMI

⁴ calculated as milk N/N intake*100

Animals fed the HIGH uN diet had 1.6 and 1.9 kg/d lower milk yield and ECM, respectively compared with those fed the LOW uN diet. The observed milk yield in this experiment was not different between treatments during the covariate period when all the cattle were fed the LOW uN diet and averaged 43.2 kg/d. After one week of feeding the experimental diets, milk yield for the cattle fed the HIGH uN treatment was 1.6 kg per cow per day lower than for the cattle fed the LOW uN treatment and this difference was maintained over the entire experimental period (Figure 2.2). Milk true protein yield for the cattle fed the LOW uN treatment was 30 g/d higher compared to the cattle fed the HIGH uN treatment ($P < 0.03$). Furthermore, cattle fed the HIGH uN diet had 1.4 mg/dl lower MUN levels than the cattle fed the LOW uN diet ($P < 0.01$; Figure 2.3). Body weight change and BCS change were not different between treatments; however, cattle fed the LOW uN diet were on average 5 kg heavier than those fed the HIGH uN diet (Table 2.3). Average gross feed efficiency (**FE**) and average milk N efficiency (**MNE**; milk N / N intake * 100) in this experiment were 1.53 kg milk / kg DMI and 29.9 %, respectively, and were not different between treatments.

4.2. Model Predictions

The ME allowable milk yield predictions for both treatments were higher than observed, either total milk or ECM yield by 3.1 to 6 kg (Table 2.4). Using the detergent system to calculate uN, the model over-predicted MP allowable milk yield by 2.9 kg and 4.2 kg for the LOW uN and HIGH uN, respectively. However, using the uN data as an input in place of ADIN the model over-predicted MP allowable milk of the LOW uN treatment by 0.6 kg and under-predicted HIGH uN milk by 1.1 kg, indicating that the estimation of the uN assay was more biologically correct compared to NDIN and ADIN for predicting the protein indigestibility.

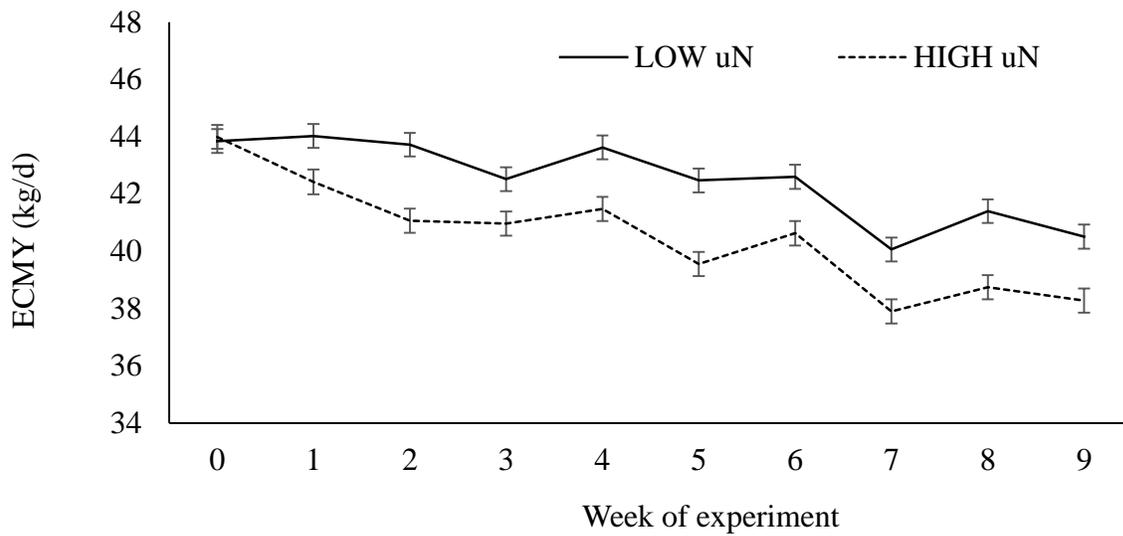


Figure 2.2. The energy corrected milk yield (ECMY) for cattle fed LOW and HIGH unavailable nitrogen diets by week of experiment. Data are reported as least squares means.

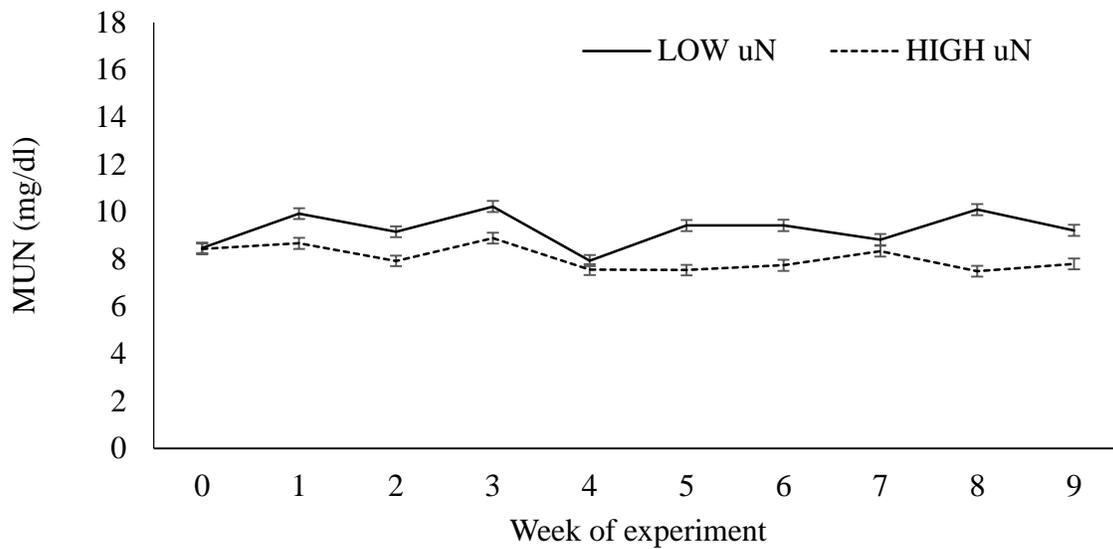


Figure 2.3. The milk urea nitrogen (MUN) for cattle fed LOW and HIGH unavailable nitrogen (uN) diets by week of experiment. Data are reported as least squares means.

Table 2. 4. The actual and energy corrected milk and the metabolizable energy (ME) and protein (MP) allowable milk for both treatments predicted by the CNCPS using the assay data of (Ross et al., 2013) to estimate unavailable nitrogen (uN), or using the original detergent system fractionation approach using acid detergent insoluble nitrogen (ADIN) as the unavailable fraction.

Item ¹	Treatment ²	
	LOW uN	HIGH uN
Actual milk, kg	42.0	40.4
Predicted energy corrected milk, kg	41.9	40.0
Predicted ME allowable milk, kg	46.2	46.0
<i>Using uN assay inputs</i>		
Predicted MP allowable milk, kg	42.6	39.3
Predicted MP supply, g	3,036	2,835
<i>Using NDIN and ADIN</i>		
Predicted MP allowable milk, kg	44.9	45.1
Predicted MP supply, g	3,105	3,144

¹ ME: metabolizable energy; MP metabolizable protein

² LOW: low uN treatment, HIGH: high uN treatment.

4.3. Economic Analysis of Digestibility

The average market value for SBM and BM in 2014 was \$466 and \$1480, respectively. On average, BM was priced 3.2 times higher than SBM. The actual market value for BM and SBM during 2014 is presented in Figure 2.4. During this period, the market value for BM fluctuated between two and four times the price of SBM.

The hypothetical increase in feed cost derived from feeding a BM with lower digestibility versus one with higher digestibility both available in the market for the same price is presented in Table 2.5. The opportunity cost was positively correlated to the market value of BM, however under all three scenarios there was an economic benefit to feeding a more digestible BM. The

increased cost is the product of feeding more of the lower digestibility BM to offset the lower digestibility.

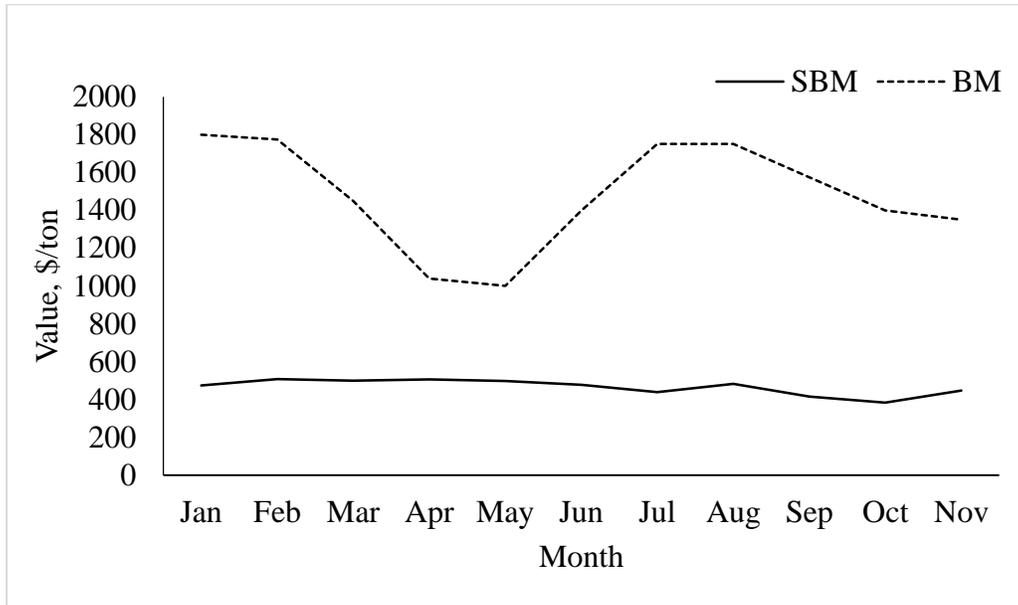


Figure 2.4. Market prices during 2014 for blood meal and soybean meal as reported by UW-Madison FeedVal 2012: http://dairymgt.info/tools/feedval_12_v2/index.php.

Table 2.5. Sensitivity analysis for feeding a lower digestible blood meal versus a higher digestible blood meal expressed in dollars per percentage point of digestibility difference.

Item,	Market Price of BM with respect to SBM		
	2x	3x	4x
Blood meal price, \$	933	1,399	1,866
Increased cost, \$/cow/day	0.20	0.29	0.39
Increased cost ¹ , \$	122	177	238

¹Increased cost of feed due to a higher inclusion of the low digestibility BM to offset the lower digestibility. Expressed as the dollar value for 1 percentage point difference in digestibility for feeding 500 cattle per month.

Assuming that the difference in digestibility between the two BM is 25%, as observed in this experiment, using the average market price for BM for 2014, the increased cost would be \$4,760 per month.

Table 2.6. Sensitivity analysis for reduced income for every percentage unit decrease in digestibility of blood meal. The revenue from feeding the higher quality blood meal is positive until the opportunity cost becomes negative.

Item,	Milk Price ¹		
	Low	Intermediate	High
Milk price, \$/cwt	11.7	17.5	25.6
Opportunity cost ² , \$/d	128	192	281

¹ Milk price calculated from the average component pricing for the CURC during 2014. Ratio between components was identical in the three scenarios reported.

² The opportunity cost corresponds to the potential increment in milk production due to feeding the more digestible BM. It is expressed as the dollar value for 1 percentage point difference in digestibility for feeding 500 cattle per month.

The opportunity cost derived from feeding a BM with unknown digestibility with an actual digestibility lower than expected are presented in Table 2.6. The sensitivity analysis indicated a positive correlation between the milk price and the magnitude of the opportunity cost.

5. Discussion

This study demonstrates there is an opportunity to balance for uN in lactating dairy cattle provided the proper information is available and all feed chemistry, and animal management and characteristics are properly characterized in a useful manner. In this study, treatments were established using two different BM that were evaluated using a new assay to predict uN in place of the traditional use of both NDIN and ADIN and the outcome suggests, at least for animal proteins and most likely low fiber feeds, that the new assay provides more biologically correct information than the detergent approach. Due to the small differences in N indigestibility, the

study design was sensitive to the predicted differences in MP supply and the cattle responded accordingly and because the inputs are readily interchangeable, it provided the opportunity to test the sensitivity of the MP predictions using the CNCPS and evaluate them post-study.

The observed differences in milk yield, ECM, milk true protein and MUN suggest that the cattle fed the HIGH uN diet had lower MP supply than the animals fed the LOW uN diet. These results were consistent with the prediction from the uN assay which indicated that the 34% uN BM would provide lower MP supply than the 9% uN BM. With the observed DMI of the initial formulated diet, the expected difference in MP supply was approximately 5.8% of the total intake or approximately 39 g of N, (244 g MP) and the initial diet was balanced for both ME and MP allowable milk, thus any deficiency in MP supply should have been apparent in some production outcome. The actual difference in predicted MP supply was 201 g/d or 32.2 g N per day which was reasonably close to the values estimated while formulating the diets.

In the study by Noftsger and St-Pierre (2003), they utilized the Three-step Assay of Calsamiglia and Stern (1995) to estimate the intestinal digestibility of animal proteins used in their study and indicated that their BM and poultry meal had greater than 90% intestinal digestibility and the feather meal utilized was greater than 85% intestinal digestible according to the assay. These values are similar to the high digestibility BM utilized in this study at 91% digestibility, whereas the low digestibility BM was approximately 66% digestibility. The feather meal digestibility described in Noftsger and St-Pierre (2003) was many units higher than most if not all feather meals analyzed in our lab using the method of Ross (2013; unpublished results). Noftsger and St-Pierre (2003) observed a significant difference in milk yield utilizing the ingredients selected for high intestinal digestibility, however it is difficult to compare directly with the outcome of this study due to the stage of lactation of the cattle used in their study and

also because of the MP predictions supplied. The differences in MP supply in this study are approximately 181 g with a milk yield difference of 2 kg whereas in the study of Noftsker and St. Pierre the MP differences were approximately 277 g, with a milk yield difference of approximately 6 kg. In the current study, diets were balanced for methionine so that should not have been a limiting AA, however the cattle were later in lactation, averaging 167 DIM over the treatment period and nutrients were being partitioned to body reserves and not milk yield, thus the difference in observed efficiencies between the two studies.

No differences in DMI and N intake were expected since the diets were formulated using the same ingredients to be iso-nitrogenous, iso-caloric, iso-NDF and iso-forage NDF. Higher DMI than reported in mid-late lactation cattle (Kalscheur et al., 1999, Groff and Wu, 2005, Alstrup et al., 2014) was attributed to the higher digestibility of the BMR corn silage compared with conventional corn silage (Hall and Mertens, 2012). The formulated protein concentration of the diet was not different from that reported by (Broderick, 2003) with 15.1 % DM; however, the average N intake in this study was 158 g/d higher due the greater DMI observed on this experiment.

For experiments evaluating MP supply, the treatment diet that is formulated to meet the MP requirement or to be first limiting will not be in a sensitive range of supply as the DMI increases unless the ME allowable milk and milk yield increases concurrently, or the protein concentration is decreased appropriately; otherwise interpretation of study results will be incorrect. This was a possibility in this study since cattle were in mid- to late-lactation and as they progressed in DIM, their MP requirements decreased with the change in milk yield, thus adjustments to the CP content of the diets needed to be made in order to ensure the cattle were in a sensitive range for the differences in digestibility of the BM ingredients. This is especially significant when

attempting to evaluate nutrition models to understand what is first limiting or how well the model might predict the first limiting nutrient. In many cases the MP supply increases in a collinear manner with ME intake due to increased microbial yield and passage of undegraded feed protein from the rumen. This results in situations, especially with data from published studies, where it is difficult to detect whether protein or energy is first limiting because not enough information is provided in the publications. For example, BW and BCS change along with pregnancy require protein and if the data are not appropriately described over time it is difficult to estimate the partitioning of protein to those other productive uses.

The average feed efficiency (FE) and milk N efficiency (MNE) observed in this study were lower than reported by Noftsker and St-Pierre (2003). This can be explained by the differences in the stage of lactation of the cattle in both experiments. The FE observed in the current study was similar to that reported by Broderick (2003) in mid-lactation cattle (on average 127 DIM at the beginning of the experiment) and fed 15.1 % CP diets with a similar forage base. The MNE in this study was higher than what Kalscheur et al. (1999) reported for mid-lactation cattle and is most likely due to the higher CP levels fed in that study.

5.1. Model Prediction Evaluation

The model evaluation consisted of testing if the uN value from the indigestibility assay could improve the prediction of MP allowable milk using the data from this study. Given that the diet composition other than BM were similar, it was assumed that the observed differences in cattle performance between treatments was due to the effect of the BM. There were no differences in the prediction of ME allowable milk using the two approaches and demonstrates that the difference in MP supply had no impact on the energy predictions. Furthermore, the ME predictions indicate that there was adequate energy for both treatments and that MP supply was

first limiting relative to ME for both treatments, thus the ME predictions were irrelevant to the interpretation of the study.

The difference in the uN consumption between treatments established the difference in digestibility being tested in this experiment. A 38 g difference in uN consumption for an average 668 g/d N intake represents a 5.9 % difference in N digestibility. The magnitude of the difference was smaller than originally intended for the experiment because a BM with higher uN could not be sourced at the beginning of the experiment. We have measured several samples of BM with uN values in excess of 40% of the total N (unpublished data), but were not able to secure adequate amounts of those to conduct the study.

In the CNCPS evaluation it is apparent that the feed chemistry described through the detergent system was not appropriate to allow the model to predict the most limiting nutrient in this comparison using BM as the treatment (Table 2.4). When the uN data were used to describe the chemistry of the BM, the model provided an acceptable and realistic prediction of the most limiting nutrient. It was also important to recognize that an accurate and complete description of the animal characteristics was important to make this evaluation and in the absence of that information, the model would not accurately predict the MP allowable milk difference. For the cattle inputs, the expected BW change based on the target growth approach was used and the BCS change was also inputted over the period of the 9-wk study period, thus this accounted for the distribution of nutrients to other productive uses and not just milk output. Using both inputs as measured is not appropriate due to the positive relationship between BW and BCS and would over-estimate the nutrients being partitioned to tissues.

5.2. Economic Analysis of Digestibility

The results of this analysis describe the potential economic cost of feeding lower digestible BM and the potential loss associated with utilizing BM with unknown digestibility. The magnitude of the cost/loss, depends on the digestibility of the BM utilized with respect to the digestibility of another BM or alternative protein source with a similar market value. Knowing the actual digestibility of the BM allows a more efficient use of this protein supplement by utilizing less BM when this has a high digestibility. Assuming that the more digestible BM was not available or was more expensive, knowing the actual digestibility would determine how much more BM needs to be fed to maintain the desired cow performance. In the second case, the cost of including more BM would be compensated by sustained production. Using the actual cost of the digestibility analysis (\$200 / sample) and assuming a worst case scenario (under the conditions of the analysis) where the price of BM was two times the cost of SBM and the price of milk was \$11.7, a minimum of 2% difference in digestibility is required to pay for the cost of the analysis, any further differences represent increase profits for the producer. Further this analysis indicated that producers could pay significantly more for the BM if they knew the digestibility.

6. Conclusions

The prediction of the uN assay for indigestible N, when applied to lactating dairy cattle demonstrated significant differences in milk and milk component yield and suggest that the uN assay results can be a useful tool describe protein indigestibility among non-forage feeds. Further, the assay values can be used in place of values like ADIN within the structure of the CNCPS or other formulation models, provided the appropriate digestibilities have been described

also and using the uN from the *in vitro* N indigestibility assay increases the accuracy of the MP allowable milk predictions in the CNCPS. According to this analysis, feeding a higher digestible BM both reduced the cost of feed and increased revenue derived from milk sales in comparison to utilizing lower digestibility BM.

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CHAPTER 3

SUMMARY AND SUGGESTIONS FOR FUTURE STUDIES

The experiment reported in Chapter 2 was designed to evaluate both the outcome of the IVNIDA and the capability of the CNCPS using that information as an input for uN to predict cattle performance in high producing dairy cattle. Two iso-nitrogenous, iso-caloric and iso-NDF diets were fed to mid to late lactation dairy cattle to compare the impact of N availability on milk yield. Treatment differences in N availability were obtained using two blood meals (**BM**) with different uN (% of total N), 9 and 34%. Higher milk yield, energy corrected milk (ECM) and milk urea nitrogen (MUN) observed in this study for the low uN treatment supported the results from the IVNIDA which indicated that the cattle fed the high uN diet had a lower metabolizable protein (MP) supply than the cattle fed the low uN diet. An evaluation using the uN values in place of ADIN in the structure of the CNCPS demonstrated that MP allowable milk using the uN values from the *in vitro* uN assay increased the accuracy of the prediction and enabled the model to predict the first limiting nutrient. Further experimentation should be conducted using early lactation cattle to determine if the efficiency of N use observed in this experiment can be improved during that stage of lactation. Analyses of the digestibility of essential amino acids should allow a better understanding of how much can N in the diet be reduced without affecting milk yield.

Also, the IVNIDA was developed to be a commercially viable technique to provide a more physiological estimate of the portion of feed N that is not available to the cow. This assay is a refinement of existing *in vivo* and *in vitro* methods to determine N digestibility in cattle. It simulates the rumen fermentation, gastric digestion and intestinal digestion, aiming to reflect the conditions of the gastrointestinal tract of the cow *in vitro*. The assay appears to capture a greater

portion of the variation in the uN content of concentrate feeds than calculated according to the CNCPS based on the detergent system.

A preliminary analyses of the between-run variation in the IVNIDA was done using the blanks and controls which are included in all runs of the IVNIDA (Table 3.1). Future runs could be designed to evaluate the within and between-run variation in the IVNIDA utilizing high and low NDF feeds. Further studies should focus on analyzing the impact of the *in vitro* fermentation on uN determination. Preliminary data from our lab suggests that the current duration of the *in vitro* fermentation (16 h) may be contributing to an overestimation of uN for forages and high NDF containing feeds due to inadequate digestion of cellulosic material before leaving the ruminal *in vitro* step. For high forage feeds, a longer fermentation time is likely needed to obtain more physiologically relevant results – something closer to 30 hr to represent the normal turnover time of forage from the rumen.

Table 3.1. Unavailable nitrogen in corn silage NDF with rumen fluid, corn silage NDF without rumen fluid, blank, positive control and negative controls as determined by the *in vitro* N indigestibility assay.

Item	n	Mean	SD	CV
Corn silage NDF with rumen fluid, mL HCl	3	3.5	0.4	11.8
Corn silage NDF without rumen fluid, mL HCl	3	2.0	0.4	21.8
Blank, mL HCl	3	1.0	0.3	30.4
Positive control blood meal, uN g/g N	3	11.5	2.8	24.4
Negative control blood meal, uN g/g N	3	92.0	2.3	2.4

Further, our data suggests that for some non-NDF containing feeds like BM, the *in vitro* fermentation step may not be absolutely required but determining which feed responds appropriately to skipping that step would be difficult if not impossible (Table 3.2).

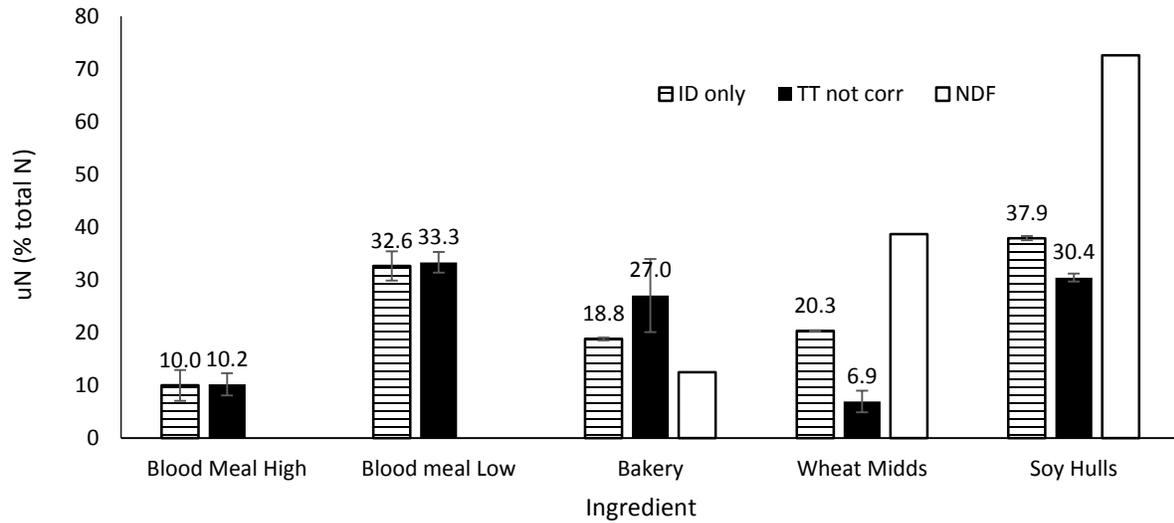


Figure 3.1. Unavailable N on concentrates determined after running the complete *in vitro* N indigestibility assay procedure or after running only the gastric and intestinal digestion.

The IVNIDA could also benefit from analyzing the impact of the microbial contamination correction on individual feedstuffs. Comparing the impact of applying the microbial correction based on the NDF content of the feed versus a common correction applied to all samples equally, should increase the precision of the technique in determining uN in high forage feeds.