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# Nutrient Utilization, Lactational Performance, and Profitability of Dairy Cows by Feeding Protein Supplements in High-Forage Lactation Diets

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# NUTRIENT UTILIZATION, LACTATIONAL PERFORMANCE, AND PROFITABILITY OF DAIRY COWS BY FEEDING PROTEIN SUPPLEMENTS IN HIGH-FORAGE LACTATION DIETS

by

Kathryn Neal

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# ABSTRACT

Nutrient Utilization, Lactational Performance, and Profitability of Dairy Cows by Feeding Protein Supplements in High-Forage Lactation Diets

by

Kathryn Neal, Master of Science

Utah State University, 2014

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Due to the increasing cost of soybean meal and concerns of excess N being excreted into the environment, new protein supplements have been developed. Two products that have shown potential in increasing N utilization efficiency are slow release urea (**SRU**; Optigen) and ruminal escape protein derived from yeast (**YMP**; DEMP). The objective of this study was to assess the effects of feeding these 2 supplements in high-forage [(54% of total dietary dry matter (**DM**)] dairy diets on nutrient utilization, feed efficiency, lactational performance of dairy cows, and their impacts on income-over feed costs. Twelve multiparous dairy cows were used in a triple 4 × 4 Latin square design with one square consisting of ruminally cannulated cows. Treatments included: 1) control, 2) SRU-supplemented total mixed ration (**TMR**, **SRUT**), 3) YMP-supplemented TMR (**YMPT**), and 4) SRU and YMP-supplemented TMR (**SYT**). The control consisted only of a mixture of soybean meal and canola meal (**SBMCM**) in a 50:50 ratio. The SRU and

the YMP were supplemented at 0.49% and 1.15% DM, respectively. The experiment consisted of 4 periods lasting 28 d each (21 d of adaptation and 7 d of sampling). Cows fed YMPT and SYT had decreased intake of DM, and all supplemented treatments had lower crude protein intake compared to those fed the control. Milk yield tended to have the greatest increase in YMPT compared with the control (41.1 vs. 39.7 kg/d) as well as a tendency for increased milk fat and protein yields. Feed efficiencies based on yields of milk, 3.5% fat-corrected milk, and energy-corrected milk increased at 10-16% due to protein supplementation. Cows fed with protein supplements partitioned less energy toward body weight gain, but tended to partition more energy toward milk production. Efficiency of use of feed N to milk N increased by feeding SRUT and YMPT, and milk N-to-manure N ratio increased in YMPT. Cows fed SRUT or YMPT tended to improve income-over feed costs. Overall results from this experiment indicate that replacing SBMCM with SRU and YMP in high-forage dairy diets can be a good approach to enhance dairy profitability through improved nutrient utilization efficiencies by lactating dairy cows.

(103 pages)

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need to be successful.

Katie Neal

# CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

## LIST OF ABBREVIATIONS

AA = amino acid

ADF = acid detergent fiber

AH = alfalfa hay

BW = body weight

CHO = carbohydrate

CM = canola meal

CP = crude protein

CRU = controlled release urea

CWT = hundredweight

DEMP = dietary escape microbial protein

DM = dry matter

DMI = dry matter intake

EAA = essential amino acid

EMPS = efficiency of microbial protein synthesis

ENU = efficiency of nitrogen utilization

F:C = forage-to-concentrate ratio

FCM = fat corrected milk

IOFC = income over feed cost

LRCpH = Lethbridge research centre ruminal pH measurement system

MCP = microbial crude protein

ME = metabolizable energy

MkN:MaN = milk nitrogen-to-manure nitrogen ratio

MUN = milk urea nitrogen

N = nitrogen

NDF = neutral detergent fiber

$NE_L$ = net energy lactation

NFC = non fiber carbohydrates

$NH_3$-N = ammonia nitrogen

NPN = non-protein nitrogen

NRC = national research council

OM = organic matter

PD = purine derivatives

peNDF = physically effective neutral detergent fiber

RDP = ruminally degradable protein

RUP = ruminally undegradable protein

SBM = soybean meal

SBMCM = soybean meal canola meal 50:50 mix

SRU = Optigen

SRUT = total mixed ration with Optigen

SYT = total mixed ration with Optigen and DEMP

TMR = total mixed ration

TP = true protein

UN:FN = urinary nitrogen excretion-to fecal nitrogen excretion ratio

VFA = volatile fatty acids

YMP = dietary escape microbial protein

YMPT = Total mixed ration with DEMP

# INTRODUCTION

The dairy industry has been challenged to meet increasing public demand for environmentally responsible nutrient management, in addition to maintaining a high degree of herd health, productivity, and profitability. Consequently, one of the major objectives of the U.S. industry system is to develop a sustainable farming system with environmentally-friendly production management. Efficient use of nutrients is one of the major assets of sustainable agricultural production systems, because inefficient nutrient use not only results in excessive and potentially harmful losses to the environment, but also affects economic performance (Oenema and Pietrzak, 2002). For instance, in ruminants fed high-quality fresh forage diets, most proteins are rapidly degraded releasing between 56 and 65% of the nitrogen (**N**) in the rumen during rumination. Consequently, large losses of N occur (25-35%) when ammonia is absorbed in the rumen (Min et al., 2000). Reduction of this wasteful loss of ammonia and the resulting optimization of animal feeding and management have been described as a key strategy for the reduction of nutrient excretion in manure (CAST, 2002; Cerosaletti et al., 2004; Ipharraguerre and Clark, 2005a). The correct match between the quantity and quality of nutrients required by the animal, together with an increase in animal productivity, helps improve the efficiency of nutrient use for milk production and the reduction in nutrient excretion (Rotz, 2004). In the long run, as environmental certification becomes a more and more important (if not mandatory) global trading factor, compliance with worldwide nutrient management standards will be crucial for the U.S. dairy industry to remain competitive. Consequently, improving nutrient utilization efficiency, particularly N on

dairy operations is an imminent task for U.S. dairy operation systems.

Feeding high-forage diets to lactating dairy cows provides many benefits including increased digestibility when high-quality forages are included as well as decreasing the risk of ruminal acidosis and other health concerns. The Intermountain West (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and Nevada) is unique in that typical lactation dairy diets contain relatively greater amounts of alfalfa hay (**AH**); baled AH commonly provides 50 to 75% of the dietary forage with total forage levels averaging 45 to 55% of dietary dry matter (**DM**; Holt et al., 2010). With optimal growing conditions, it is not uncommon to feed high-quality AH with at least 21.3 and 38.3% DM of crude protein (**CP**) and neutral detergent fiber (**NDF**) on average, respectively (Holt et al., 2013). Although feeding AH provides CP and enough forage NDF to support potential milk production, its protein is extensively broken down in the rumen by microbes, resulting in less than optimal microbial protein (**MCP**) synthesis, increased energy costs to convert ruminal ammonia-N (**$NH_3$-N**) to urea, and excess N excretion into the environment. Because of the poor utilization of CP in alfalfa-based diets by the animal (Castillo et al., 2001), there is a need to find strategies to improve nutrient utilization including feed N in lactation rations, especially in this region.

Microbial protein is the main source of protein for dairy cows providing, 50 to 80% of total absorbable protein with higher concentrations of Met and Lys, the two most limiting amino acid (**AA)** for milk production (NRC, 2001). It is important to optimize MCP synthesis by meeting the protein requirement of the cow with the lowest dietary CP input, while still maintaining the best ratio between rumen degradable protein (**RDP**) and

rumen undegradable protein (**RUP**) to support milk production and optimize N utilization efficiency (Agle et al., 2010). Alfalfa hay alone is unable to meet these requirements, and therefore must be supplemented with other protein sources. Soybean meal (**SBM**) is a common protein source, but because of its high degradability in the rumen as well as its increasing cost, alternative protein supplements have been developed. Urea is a chemically synthesized non-protein N (**NPN)** that can be used to supplement dietary CP, but is quickly and extensively broken down in the rumen increasing the ammonia concentration rapidly. Alltech (Nicholasville, KY) has developed 2 commercial protein supplement products: 1) slow release urea (**SRU**; Optigen) which is urea coated in vegetable oil, slowing its release of ammonia, and 2) yeast-derived MCP (**YMP**; DEMP) with an AA profile that more closely matches the composition of ruminal MCP and presumably flows with the liquid phase of the rumen allowing for increased absorption of AA in the small intestine (Sabbia et al., 2012). In a recent study, when SRU was added to high-forage dairy diets, consisting of 23.7% corn silage and 27.7% alfalfa silage of total dietary DM where SRU replaced SBM, there was an increase in milk yield compared with a control diet (35.9 vs. 35.4 kg/d; Inostroza et al., 2010). However, when the rumen reaches the point of $NH_3$-N overflow, adding more RDP will not increase MCP synthesis (Satter and Slyter, 1974). Instead, if a high-quality RUP is supplied, and there is already a sufficient amount of RDP, the amount of AA absorbed in the small intestine can be increased, which supports milk production (Santos et al., 1998; Kalscheur et al., 2006). For example, Sabbia et al. (2012) reported improved milk and total solids production, when SBM was replaced with YMP in high-forage dairy diets. Therefore, SRU and YMP

have a high potential to improve nutrient utilization and lactational performance when supplemented in high-forage lactation diets consisting of AH.

The research presented in this thesis will test the hypothesis that adding SRU and/or YMP to a high-forage lactation diet consisting of a high dietary concentration of AH will improve N utilization efficiency, enhance lactational performance, and decrease N excretion into the environment. Additionally, income-over feed costs (**IOFC**) will be discussed with the results of milk and milk component yields and feed efficiencies.

# REVIEW OF LITERATURE

Increasing the efficiency of conversion of feed N into milk by dairy cows is an integral part of the efforts to maintain or increase dairy production, while decreasing negative environmental impacts by dairy operations. It is the purpose of this review to examine the flow of protein metabolism and the efficiency of high-forage diets, and how these metabolic events can change in response to protein supplementation. In addition, the environmental impacts due to protein supplementation in lactation dairy diets will be discussed.

## Metabolism of Nitrogen

Nitrogen metabolism in ruminants is a complicated process involving many mechanisms and pathways, illustrated by Figure 1 (Wattiaux, 1998); overall ruminal N metabolism is broken down into 2 processes: protein degradation and MCP synthesis (Bach et al., 2005). Because of extensive microbial fermentation and modification in the rumen, the protein that is absorbed in the small intestine is much different than the original feed protein fed to the animal. There are 2 types of feed proteins in ruminant diets. Rumen degradable protein is protein that is broken down in the rumen into peptides, AA, and $NH_3$-N to support MCP synthesis and is provided in 2 forms, NPN or true protein (NRC, 2001; Bach et al., 2005). Rumen undegradable protein escapes microbial fermentation, minimizing degradation in the rumen (NRC, 2001). Endogenous protein is also an important source of protein for ruminants which includes sloughed off epithelial cells from the gastrointestinal tract as well as enzymes secreted in the

abomasum (Tamminga, 1992). NRC (2001) defines metabolizable protein as the true protein or AA absorbed in the small intestine, which includes MCP, RUP, and endogenous protein. Post-ruminally, metabolizable protein is absorbed in the small intestine, providing the animal with the AA necessary to support maintenance, growth, pregnancy, and milk production (NRC, 2001). If metabolizable protein exceeds the requirements of the animal, then it will be excreted into the milk or urine in the form of urea (Van Soest, 1994). The goal of N metabolism in ruminants is to optimize MCP synthesis by providing sufficient RDP and supplementing with a high-quality RUP to support greater production and to decrease the losses of N into the environment.

**Protein Degradation in the Rumen**

All protein degradation in the rumen occurs through enzymatic activity of the microbes including bacteria and protozoa (NRC, 2001). Bacteria make up the greatest proportion of microbes in the rumen; 40% of those bacteria are known to have proteolytic activity (Wallace et al., 1997), and most of these bacteria act on the cell surface (Kopecny and Wallace, 1982). In order for protein degradation to occur, the microbe must come into contact with the protein, making it the initial step in protein degradation (Wallace, 1985). The rate and extent of proteolysis not only influences MCP synthesis by providing the $NH_3$-N, AA, and peptides necessary, but it also affects the quantity and quality of RUP that reaches the small intestine (Stern et al., 1994). Many factors are involved in determining the rate and extent of protein degradation including, but not limited to, type of protein, ruminal passage rate, ruminal pH, and substrate (Bach et al., 2005).

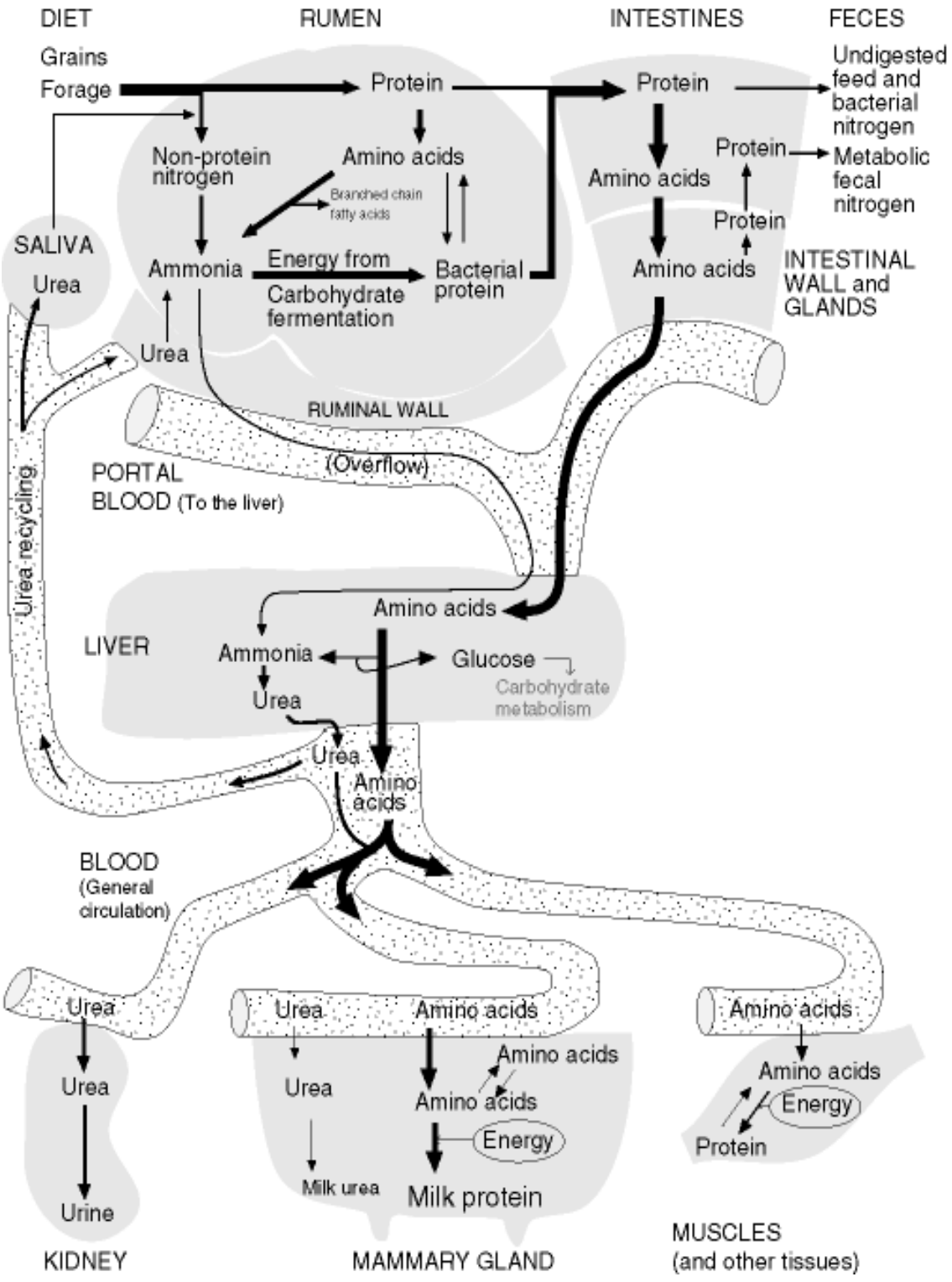


**Figure 1.** Nitrogen metabolism in dairy cows (Wattiaux, 1998)

Solubility plays an important role in degradability of a protein (Bach et al., 2005), but soluble proteins from different feeds are degraded at very different rates (Hedqvist and Udén, 2006), indicating that structure as well as solubility affects protein degradation in the rumen (NRC, 2001). Protein solubility is usually measured chemically with solvents, but this not equivalent to protein degradation in the rumen for several reasons (NRC, 2001). Solubility is affected by factors like the type of buffer used, pH, temperature, and extraction time (Crawford et al., 1978), demonstrating that there is a poor relationship between protein degradation rate in the rumen and N solubility (NRC, 2001). The structure of the protein is important in determining the degradability of the protein. The 3-dimensional structure and chemical boding of the protein affect how the microbes are able to access the protein, which may be the most important factor, affecting the rate and extent of protein degradation in the rumen (NRC, 2001).

Passage rate influences protein degradation, having an inverse relationship (Ørskov and McDonald, 1979). There are several factors that affect passage rate including dry matter intake (**DMI**). When DMI is increased, the passage rate is also increased, which in turn can decrease digestion in the rumen (Shaver et al., 1988). Passage rate of feedstuff from the rumen is affected by particle size of the diet demonstrated by Yang et al. (2002) who fed diets consisting of 60% barley-based concentrate and 40% of alfalfa silage and hay either chopped or ground to change particle size. The authors found that when forage particle size was increased, digestibility of N was improved due to the increased retention time in the rumen (Yang et al., 2002). Krämer et al. (2013) determined that forage type has more influence on passage rate than the forage-to-concentrate (**F:C**) ratio of the feed.

When passage rate is increased, there is not enough time for the microbes in the rumen to attach to the feedstuffs and degrade the protein.

The composition of the diet as well as the pH influence protein degradation in the rumen (Bach et al., 2005). Rations higher in forage have shown to have greater protein degradation (Church, 1988). This could be due to several factors including increased ruminal pH with higher forage diets. The optimal ruminal pH for proteolytic enzymes is between 5.5 and 7.0 (Kopecny and Wallace, 1982), and microbes that produce proteolytic enzymes tend to be more prevalent in more neutral ruminal pH (Church, 1988). Cardozo et al. (2000, 2002) studied high-forage compared with high-concentrate diets in a dual flow continuous culture system and found that protein degradation was reduced in both types of rations. From this it is understood that diet composition as well as pH influence the microbial population in the rumen.

Effects of diet composition and ruminal pH on protein degradation may be influenced by nutrient interactions and the predominant microbial population in the rumen (Bach et al., 2005). Protein degradation not only depends on the activity of proteolytic bacteria and enzymes, but on other enzymatic activity as well, including cellulase and amylase. Assoumani et al. (1992) added amylase to cereal grains which resulted in increased protein degradation up to 20%. The protein in a feedstuff may be trapped within the fiber and will not be degraded unless the fiber is degraded first. Cellulolytic microbes are more prevalent in higher forage diets, leading to greater degradation of cellulose and allowing easier access for proteolytic microbes to attach to the protein (Church, 1988). Debroas and Blanchart (1993) discovered that protein that was bound by NDF was not degraded

until degradation of cellulose by cellulolytic bacteria occurred and allowed access for proteolytic bacteria. When cellulase was added in an in vitro experiment, protein degradation was increased from 42.4 to 53.1%, confirming the results discussed above (Kohn and Allen, 1995). Endres and Stern (1993) observed that when pH was decreased from 6.3 to 5.9 in higher concentrate diets, the cellulolytic bacteria population was reduced by about 50%, while counts of proteolytic bacteria were not affected. The reduction in cellulolytic bacteria in higher concentrate diets may lead to a decrease in protein degradation, although the number of proteolytic bacteria does not change. It can be concluded from these results that the diet composition and pH change the microbial population in the rumen, influencing protein degradation.

Protozoa play a major role in N metabolism as well, but in a different way than bacteria do. Protozoa ingest their feed instead of forming a complex with it like bacteria (NRC, 2001). Bacteria are protozoa's main source of protein, and because protozoa act in this way they are more able to degrade insoluble feed proteins (Jouany and Ushida, 1999). There are 2 main classes of protozoa in the rumen, entodiniomorphids and holotrich. While entodiniomorphids attach to fibrous material in the rumen, holotrich can travel from the reticulum to the rumen (Abe et al., 1981). *Entodinium sp.* have been found to make up to 80 to 98% of the total protozoal population (Dehority, 2003), compensating for its low activity and allowing it to have the greatest effect on bacterial predation and degradation (Belanche et al., 2012). Belanche et al. (2012) performed a study to determine which group of protozoa had the most influence on protein degradation. The authors found that in conventional livestock diets 70 to 75% of bacterial

protein was degraded by *Entodinium sp* (Belanche et al., 2012), which was in agreement with previous results. Once protein is ingested by protozoa, it is degraded to peptides and AA, where the AA can be integrated into protozoal protein (NRC, 2001). Another difference between protozoa and bacteria is that protozoa are not able to synthesis AA from $NH_3$-N, but act as $NH_3$-N transporters (Jouany and Ushida, 1999). The last different between protozoa and bacteria is that protozoa release peptides, AA, and peptidases in the rumen (NRC, 2001), resulting from autolysis and significant secretory processes (Dijkstra, 1994), and creating a high rate of protozoal protein recycling in the rumen (Punia et al., 1992).

**Synthesis of MCP**

When energy is available in the rumen, the end products of protein degradation (peptides, AA, and $NH_3$-N) can be used to synthesize MCP (Bach et al., 2005). Microbial protein provides between 50 and 80% of the total protein in dairy cows and is a high quality AA source including higher levels of Met and Lys, the 2 most limiting AA for milk production, and high digestibility (80%; NRC, 2001). The AA composition of MCP closely matches the requirements for lactation and growth in ruminants with Lys and Met in MCP averaging 15.8 and 5.2%, respectively (Clark et al., 1992; NRC, 2001). There is evidence suggesting that increasing amounts of Lys and Met may contribute to increased milk fat concentration. It is still unclear why AA affect milk fat synthesis, but it is known that around 50% of milk fat is synthesized de novo in the mammary gland from precursors like Met. This may be due to the influence that Met has on the synthesis of short- and medium-chain fatty acids, or the role that AA play in the hepatic synthesis of

chylomicrons and very-low density lipoproteins (NRC, 2001; Guretzky et al., 2006). In addition, Met is required as a methyl donor in the synthesis of choline, which contributes to the formation of phospholipids (NRC, 2001). Consequently, if Met supply is insufficient, choline synthesis will be depressed, possibly leading to decreased milk and milk fat yields (NRC, 2001; Guretzky et al., 2006; Wang et al., 2010).

Ruminal bacteria are defined by their substrate preference, and their growth is influenced by many factors. Amylolytic bacteria prefer non-structural carbohydrates (**CHO**), while cellulolytic bacteria prefer structural CHO. They also prefer different N sources. Cellulolytic microbes prefer $NH_3$-N and have a low maintenance requirement, while amylolytic microbes prefer $NH_3$-N, peptides, and AA and hive a high maintenance requirement because of their rapid growth (Russell et al., 1992). When typical concentrations of AA and peptides are found in the rumen, approximately 80% of microbial cell N is derived from $NH_3$-N (Bach et al., 2005). Dairy cattle are fed mixed rations, allowing for structural and non- structural CHO as well as true protein and NPN degraded in the rumen to maximize MCP synthesis (Brito et al., 2007). Atasoglu and Guliye (2004) suggested that Lys can limit microbial growth of microbes in the rumen, concluding that supplying specific AA might result in greater MCP synthesis.

Concentration of ruminal $NH_3$-N affects MCP synthesis, but research indicates variable results in what concentration will maximize MCP growth. Satter and Slyter (1974) performed a continuous culture study and concluded that ruminal $NH_3$-N concentrations of 5 mg/100 mL were required for maximal efficiency of MCP synthesis. Boucher et al. (2007) observed when ruminal $NH_3$-N concentrations were above 13

mg/100 mL, MCP synthesis was decreased, and MCP synthesis was maximized when ruminal $NH_3$-N concentrations were between 11 and 13 mg/100 mL in corn silage-based diets only differing in the amount of urea fed to lactating cows, with concentrations of RDP at 10.0 and 10.8% of the diet DM (0.3 and 0.6% urea of diet on a DM basis, respectively). Similarly, Reynal and Broderick (2005) fed cows with increasing amounts of RDP (10.6 to 13.2% RDP of DM in the diet) by supplementing different protein sources (solvent and lignosulfonate-treated SBM and urea). The authors found that MCP yield and efficiency increased linearly with increasing RDP percentage and ruminal $NH_3$-N concentration up to 12.3 mg/100 mL in the 13.2% RDP ration (Reynal and Broderick, 2005). The optimal ruminal $NH_3$-N concentration is still unclear and may change depending on specific situations. In the same study by Reynal and Broderick (2005), increasing dietary RDP also resulted in increased free AA concentration in the rumen, suggesting that MCP synthesis is stimulated by AA and peptides. The authors also concluded that the RDP provided to the animal must consist of NPN as well as true protein (Reynal and Broderick, 2005). There is not a specific AA requirement for MCP growth, but it has been suggested that some AA may be more limiting to MCP synthesis than others (Atasoglu and Guliye, 2004).

The supply and type of energy available in the rumen is also critical in MCP synthesis (Bach et al., 2005). Stern and Hoover (1979) stated that starches or sugars that are more readily fermentable CHO are more effective in promoting microbial growth compared with other CHO sources, such as cellulose. Not only is providing enough energy and in the right form, it is also important to match the rate of degradation with that of the N

supply. If the rate of protein degradation exceeds CHO fermentation or vice versa, MCP synthesis can be decreased (Nocek and Russell, 1988). Sannes et al. (2002) found that including sucrose in the diet decreased MCP synthesis, but concluded that this was due to a limited RDP supply, limiting the concentration of $NH_3$-N in the rumen. Synchronizing N and CHO in the rumen has provided variable results due possibly to the wide variety of the microbes in the rumen; rations may by synchronized for one subpopulation but not synchronized for another (Bach et al., 2005). In addition, N recycling that occurs in the rumen that can stimulate MCP synthesis in situations when N is not well synchronized has to be taken into account (Bach et al., 2005).

Efficiency of MCP synthesis (**EMPS**) is defined as grams of microbial N per unit of rumen-available energy (true organic matter or CHO fermented; Bach et al., 2005). Factors such as ruminal pH and feed intake influence EMPS. As ruminal pH decreases in the rumen, EMPS is increased. Decreased ruminal pH is often associated with increased organic matter fermentation in the rumen resulting in increased MCP synthesis (Hoover and Stokes, 1991) and EMPS. When feed intake is increased, substrate availability for microbial fermentation is increased, resulting in improved MCP synthesis (Broderick, 2003). Also, when feed intake is increased, passage rate of the feed increases, leading to more undigested feed particles with microbes attached exiting the rumen (Van Soest, 1994). Increased passage rates decrease the opportunity for bacterial predation by protozoa, increasing the flow of MCP to the small intestine (Firkins et al., 1992) and improves EMPS.

Efficiency of MCP synthesis has its limitations, because although it is able to predict

how much energy is used for N deposition in microbes, it us unable to indicate how much N is being used by the microbes (Bach et al., 2005). To measure the amount of N the microbes capture, efficiency of N utilization (**ENU**) should be calculated. The ENU is calculated by dividing grams of N by grams of available N and multiplying it by 100 (Bach et al., 1999). As ruminal $NH_3$-N was decreased in continuous cultures, ENU was increased, resulting in a high correlation between the two ($R^2 = 0.78$; Bach et al., 2005). Therefore, both EMPS and ENU should be considered to determine the efficiency of MCP synthesis.

**Post-Ruminal Protein Degradation and Absorption**

Protein degradation post-ruminally is very similar to that of simple stomach animals. Rumen undegradable protein and MCP will flow out of the rumen, making its way to the abomasum where protein degradation continues. The abomasum secretes gastric juices as well as acid, pepsin, and lysozyme that aid in the digestion of protein and microbial cells. The lysozyme in ruminants is distinctive from most other species because of its specific AA sequence that provides resistance from pepsin degradation (Dobson et al., 1984). Once the acidic digesta passes from the abomasum, it flows into the small intestine where it is neutralized, and further protein digestion occurs by trypsin (Van Soest, 1994). The small intestine is covered in villi, which greatly increases its absorptive capacity when the digesta comes into contact with it. The main activity of the small intestine is the absorption of AA, because only small amounts of sugars, starch, and fatty acids escape ruminal fermentation (Van Soest, 1994). Also unique to ruminants is the high activity of pancreatic ribonuclease post-ruminally that aids in the digestion of MCP (Church, 1988).

The digestion of microbial nucleic acids post-ruminally is thought to be quite high at 80% (Church, 1988). In the small intestine the metabolizable protein is absorbed and can then be used for maintenance, growth, reproduction, and production.

## High-Forage Diets

It is essential for high producing dairy cows to consume large amounts of forages that are high in digestibility (Llamas-Lamas and Combs, 1991), but feeding high-forage diets comes with many benefits as well as concerns. The optimal ruminal pH range for microbes is between 6.2 and 7.2, which rarely occurs in the dairy industry where high concentrate diets are fed to increase milk yields (Yang and Beauchemin, 2007). In the U.S., 14 to 40% of cows in high producing herds are affected by ruminal acidosis which costs over $9 million in losses each year (Oetzel et al., 1999). High-forage diets can increase physically effective neutral detergent fiber (**peNDF**) in the ration that stimulates chewing, decreasing the risk for the ruminal acidosis. Yang et al. (2001) tested diets that differed in peNDF by changing the processing of barley, F:C, and the forage particle length. The authors found decreased DMI, total tract digestibilities of DM, but increased milk fat concentration with high F:C (Yang et al., 2001). High-forage diets are also known to decrease DMI and is considered to be due to the increased rumen-filling effect (Yang and Beauchemin, 2007). Increases in milk fat concentration were probably due to the increase in acetate-to-propionate ratio (Yang et al., 2001). Cellulolytic bacteria prefer higher ruminal pH, which can lead to an increase in fiber digestion when higher forage diets are fed. Acetate is a lipogenic volatile fatty acid (**VFA**) that is known to be a

precursor for milk fat synthesis and is increased in high fiber rations (Rook and Balch, 1961). Yang and Beauchemin (2007) reported similar results when manipulating peNDF by changing forage particle length and the F:C. When F:C was increased, there was a decrease in DMI, but an increase in fiber intake and an increase in fiber digestion, resulting in an increased milk fat concentration (Yang and Beauchemin, 2007). The authors concluded that lower F:C diets were beneficial for increasing feed intake, MCP, and milk production, but did not maximize feed digestion and feed efficiency because of the increased chance of subacute ruminal acidosis (Yang and Beauchemin, 2007).

## Alfalfa Hay

With increasing feed cost due to high grain prices, it is even more important than ever to consider feeding high-forage diets. Substituting a high-quality forage like AH for expensive grain can decrease feed costs, and AH provides a cheap source of dietary protein, making it very cost-effective (Broderick, 2001). In the Intermountain West where AH is commonly grown and is of high quality, it is not uncommon to feed high-forage rations with a high concentration of AH (Holt et al., 2010).

Alfalfa hay plays an important role in lactating cow rations by providing forage as well as representing a major protein source. The N in AH is considered to consist of 10 to 20% NPN (NRC, 2001). In alfalfa, the protein is extensively degraded in the rumen, resulting in absorbable protein being the most limiting nutritive factor (Broderick, 2001). Figure 2 shows the increased degradation rate of AH compared with other legumes (Broderick et al., 2000). Less than optimal MCP synthesis can occur when feeding AH

due to the fact that it is lower in readily fermentable energy compared with concentrates, which is related to the capturing of RDP by the ruminal microbes (Broderick, 2001).

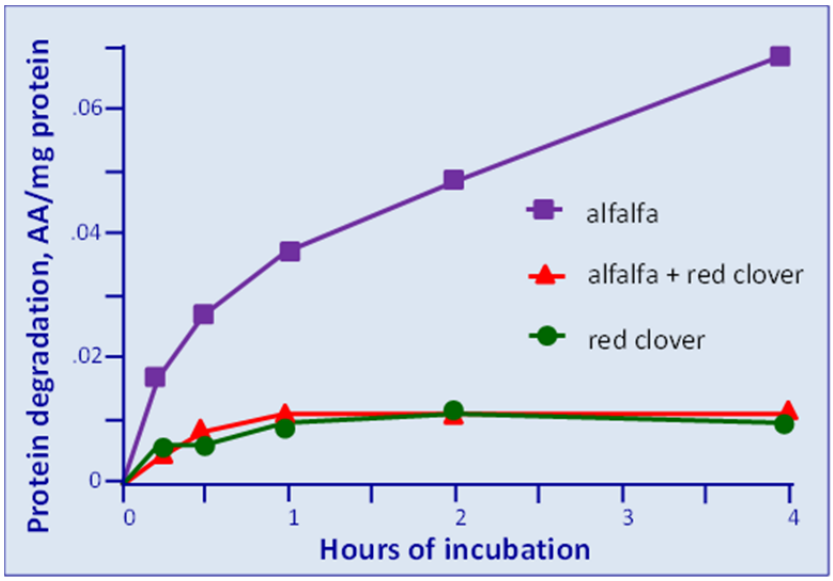


**Figure 2.** Protein degradation of different legumes (Broderick et al., 2000)

Alfalfa hay can provide NDF in the diet which is important for milk fat production. To avoid milk fat depression in dairy cows, the NRC (2001) recommends feeding at least 25% dietary NDF with 19% of dietary DM from forage NDF. It is also important to consider the particle size of the AH especially when included in high proportions of the diet. Increasing the proportion and particle size of AH in the diet can increase sorting and selection of finer particles (Leonardi and Armentano, 2003). The peNDF provided from AH in the ration increases chewing and saliva production to buffer the rumen and prevent ruminal acidosis. The optimal pH of proteolytic enzymes in the rumen ranges from 5.5 to 7.0 (Kopecny and Wallace, 1982), but degradation tends to decrease at lower pH. Alfalfa hay can provide a better fermentative environment for protein to be broken down in the

rumen.

Broderick (1985) compared feeding 60% AH to 60% corn silage on a DM basis and found decreased milk protein concentration and yield in the 60% AH treatment. The author attributed this to the increased fermentability of the corn silage and improved MCP yield (Broderick, 1985), which also resulted in decreased N losses. The authors concluded that SBM, which is also extensively degraded in the rumen (NRC, 2001), that was supplemented in the 60% corn silage diet to maintain isonitrogenous conditions had a higher utilization efficiency of the protein in the AH (Broderick, 1985). This warrants the need for more research to find protein supplements that can be included in diets with high proportions of AH to increase the utilization efficiency of the protein in AH.

## Protein Supplements

### SBM and Canola Meal

In North America, SBM is the most commonly used protein supplement in dairy rations (Borucki Castro et al., 2007) because of its high concentration of essential AA (**EAA**) compared with other oilseed meals (NRC, 2001). Soybean meal can be processed in many different ways, and this is important, because the type of process can change the RDP and RUP fractions of the SBM. One method of processing soybeans for oil is the heat generating expeller process, which does not use any organic solvents, and can increase the RUP fraction of SBM (Borucki Castro et al., 2007). According to NRC (2001), expellers SBM consists of 69.0% RUP as a percentage of CP, compared with solvent extracted SBM which averages 38.6% RUP as a percentage of CP in 50.0%

forage diets (NRC, 2001). In a dual flow continuous culture experiment, Waltz and Stern (1989) found that protein degradation in the rumen was decreased and total EAA flow was increased, when SBM was expeller processed compared with solvent extracted SBM. Another way of increasing the RUP in SBM is treating it with lignosulfate, which is a nonenzymatic browning reaction that reduces the degradability of the protein (Can and Yilmaz, 2002). As a percentage of CP nonenzymatically browned SBM consists of 79.4% RUP in 50% forage diets (NRC, 2001). The sulfate liquors used in this process are byproducts of the paper milling industry and have proved to be a source of environmental pollution (Borucki Castro et al., 2007). With raising environmental concerns, the lignosulfate process may not be the first choice for increasing the RUP fraction of SBM.

Borucki Castro et al. (2008) compared solvent extracted SBM with expeller extracted SBM and found increased plasma concentration of EAA with expeller SBM, possibly resulting in an increased intestinal supply of EAA. However, plasma concentrations of Lys and Met were similar between the two treatments (Borucki Castro et al., 2008). The authors concluded that because there was no difference in these limiting AA, it resulted in the lack of response in milk production between solvent and expeller extracted SBM (Borucki Castro et al., 2008). It is accepted that for increased milk production the AA profile of RUP should closely match that of MCP, because the AA composition of MCP closely matches that required for milk and milk protein synthesis (NRC, 2001). On average expeller and solvent extracted SBM decreased Lys and Met (13.8 and 3.2 % of total essential AA, respectively; NRC 2001) when compared to MCP which has 15.8 and 5.2% Lys and Met, respectively (Clark et al., 1992).

The cost of SBM is steadily increasing, pushing for the need to find a different protein supplement while still maintaining or improving production and decreasing feed costs. Canola meal (**CM**) is increasing in popularity in North America because of its potential to be an economical alternative to SBM (Maxin et al., 2013). Canola meal has shown promising results when replacing other protein supplements, showing responses such as increased lactational performance and N utilization efficiency (Martineau et al., 2013). Canola meal is similar to SBM in regards to its AA profile containing 13.2% of EAA of Lys and slightly higher Met at 4.39% of EAA. Canola meal is 35.7% RUP as a percentage of CP in 50% forage diets, more closely matching the RUP percentage of solvent extracted SBM.

Canola is a variety of rapeseed that contains less than 2% erucic acid in the oil and less than 30 μmol of glucosinolates/g in the meal (Newkirk, 2009). Rapeseed was not commonly fed to livestock because of its high concentration of goitrogenic compounds. Goitrogenic compounds may have negative effects on milk production because of its action of reducing the availability of iodine to the animal and decreasing the synthesis of thyroxin, which is involved in the hormonal mechanism of milk production (Martineau et al., 2013). However, glucosinolates are more harmful in monogastrics than in ruminants (Tripathi and Mishra, 2007). It was not until 1969 when a low-glucosinolate trait was discovered in a Polish spring rapeseed variety, which started an international backcrossing breeding program, leading to the low glucosinolate trait in high yielding plants (Abbadi and Leckband, 2011). Places like Canada have been growing more and more canola since the development of the low glucosinolate trait increasing the

production of CM available for animal feed. In parts of the U.S. CM has become the principle source of protein for dairy cows, because of it increasing availability and its ability to provide a high-quality protein to the animal (Mulrooney et al., 2009).

Huhtanen et al. (2011) performed a meta-analysis comparing feeding SBM, CM, and heat treated CM in grass silage-based diets and found that milk production was increased in the CM treatments. The authors reported that DMI was increased in CM compared with SBM (Huhanen et al., 2011). The increase in DMI with protein supplementation is usually attributed to increased fiber digestion because of stimulation of cellulolytic bacteria in the rumen (Oldham, 1984; Hoover, 1986). Because there were no differences in digestibilities, the increase in DMI in CM compared with SBM may be attributed to a more balanced supply of AA that improved milk production, resulting in an increased energy demand (Huhtanen et al., 2011).

When compared with SBM, CM had greater increases in milk and milk protein yields in response to greater CP intakes (Huhtanen et al., 2011). This agrees with the finding of Hristov and Huhtanen (2008) who reported that an increase of 1% dietary CP resulted in an increase of 2.8 g in milk N. Metabolizable energy supply was increased more in CM than SBM because of the increase in DMI, which increased the efficiency of CP utilization with CM (Huhtanen et al., 2011). Shingfield et al. (2003) compared heat treated CM with solvent extracted SBM in grass silage-based diets and reported increased milk and milk protein yields with the heat treated CM. Canola meal treatments increased His, Met, and branched chain AA concentrations in plasma (Shingfield et al., 2003). In grass silage-based diets, His may be the first limiting AA for milk protein synthesis

(Vanhatalo et al., 1999), which may explain the response in milk protein in the experiment by Shingfield et al. (2003). Along with the increase in milk protein, Shingfield et al. (2003) also reported decreased milk urea-N (**MUN**) concentration with CM treatments, indicating better utilization of feed N compared with SBM.

Results from a meta-analysis indicated positive responses in 4% fat corrected milk (**FCM**), energy corrected milk (**ECM**), milk protein concentration and yield, milk efficiency, and N utilization efficiency only when CM replaced protein sources other than SBM (Martineau et al., 2013). The increase in N utilization efficiency was due to positive responses in milk and protein yields possibly because of the increased supply of EAA to the small intestine when CM was included (Martineau et al., 2013). The lack of response in milk protein when CM was substituted with SBM may have been attributed to the fact that when CM substituted protein sources (corn byproducts) other than SBM their AA profile was not as good as CM or SBM (Martineau et al., 2013).

The preference of SBM over CM in diary ration is partly due to the greater CP concentration in SBM compared with CM (49.9 vs. 37.8% CP, respectively; NRC, 2001) and greater metabolizable energy content. Many feed protein evaluation systems report SBM as having increased metabolizable protein over CM on a CP basis (Huhtanen et al., 2011). This suggests that a higher dietary CP concentration is required when CM is fed compared with SBM to meet the metabolizable protein requirement, because of the decreased ruminal CP degradability which predicts a higher dietary metabolizable protein supply with SBM (Huhtanen et al., 2011). Also, many feed evaluation systems, including NRC (2001) report SBM as having a higher concentration of RUP compared with CM.

Brito and Broderick (2007) observed a 27% increase in ruminal total free AA in corn and alfalfa silage-based diets supplemented with solvent extracted SBM compared with CM. The authors attributed the increased ruminal total free AA to the increased degradation of the SBM in the rumen (Brito and Broderick, 2007). However, when Shingfield et al. (2003) fed grass silage-based diets, the authors found increased plasma concentrations of EAA in heat treated CM compared with solvent extracted SBM, not supporting the higher RUP concentration in SBM suggested by NRC (2001).

**Urea**

The discovery of the synthesis of urea by Friedrich Wöhler is known as the beginning of organic chemistry, changing the way the world thought (Kurzer and Sanderson, 1956). The production of urea occurs by reacting $CO_2$ with anhydrous ammonia under high temperatures and pressure (Gilbert et al., 2006). Seventy five percent of all urea produced is used for fertilizer, but 10% of the urea not used as fertilizer is used as a feed additive for ruminants (Gilbert et al., 2006). Urea is a NPN containing 45% N and is supplemented in dairy cattle rations, providing RDP. Ruminants are unique in that they have microbes that are able to produce enzymes allowing them to utilize feedstuff that monogastric animals may not. Urease is an enzyme that is produced by the microbes in the rumen, which breaks down urea into $NH_3$-N, allowing for ruminants to utilize it as a N source for microbial growth (Satter and Slyter, 1974).

Urea is the main NPN source used in ruminant rations (Huber, 1975), and has been fed for more than 100 years (Kertz, 2010). Feeding urea to dairy cattle appears adventitious because of its concentrated N source and decreased cost compared with

oilseed meals. Urea is 281% CP, which means a lot less has to be fed compared with SBM to meet the N requirement of the animal. This creates more room in the ration for DMI and allows for more energy to be provided. However, there are risks associated with feeding urea including toxicity, decreased DMI, MCP synthesis, and milk production. Because of urea's high degradability in the rumen, it is better utilized in rations with a higher concentrate proportion.

The liver converts ammonia to urea, but if there is excess $NH_3$-N in the rumen, the liver is unable to convert all of it, increasing the amount of ammonia in the peripheral blood resulting in toxicity (Chalupa, 1968). For $NH_3$-N to be absorbed across the rumen wall into the blood, it must be in its ionic form (ammonium; Kertz, 2010). When ruminal pH is increased, absorption of $NH_3$-N is increased, especially above pH 7.0 (Visek, 1968). Bartley et al. (1976) found a positive correlation between ruminal pH and ruminal $NH_3$-N toxicity ($R^2 = 0.32$). Higher concentrate rations typically result in lower ruminal pH, decreasing the rate of $NH_3$-N into the blood and consequently the risk of toxicity. Also, in higher concentrate rations, there is more energy available for the conversion of $NH_3$-N to MCP, especially if the concentrate provided is highly fermentable, matching the degradation rate of the urea. Once the liver detoxifies any excess ammonia by converting it to urea, it is released into the blood. This endogenous urea is then excreted into urine or milk or recycled back to the rumen via saliva or directly across the ruminal wall (Huntington and Archibeque, 2000). Around 10 to 40% of N consumed in the feed is recycled back to the digestive tract as urea from saliva or transported through the blood (Huntington and Archibeque, 2000). Diet composition, intake, and animal production

influence urea production, excretion, and recycling. Furthermore, detoxification of $NH_3$-N due to excess of urea in dairy cattle diets requires energy, reducing the available energy for production (Butler, 1998).

In areas that grow a lot of corn, feeding corn silage instead of legume silage is more commonly seen. Although corn silage has a higher digestible energy content than legume silage, it has considerably less RDP compared with other high-quality forages (Boucher et al., 2007). Supplementing corn silage-based diets with urea may be a good choice to increase the ration RDP. However, NRC (2001) recommends only feeding between 1.5 and 2.0% urea of concentrates, because at higher levels it may result in decreased DMI and lower milk yield.

There have been variable results with feeding urea, and how it affects MCP synthesis. Brito et al. (2007) supplemented high-forage diets (35% corn silage and 21% alfalfa silage) with different protein sources including urea, solvent-extracted SBM, cottonseed meal, or CM. Microbial non-ammonia N was decreased by 14% with urea compared with all other true protein-supplemented treatments, indicating that true protein is necessary to maximize MCP synthesis (Brito et al., 2007).

Broderick and Reynal (2009) found similar results when they tested the effects feeding different amounts of RDP from SBM and urea. The authors reported decreases in milk yield and milk fat and protein yields with increasing amounts of urea and concluded that this was due to a decrease in ruminal outflow of non-ammonia N, EAA, and total AA because of decreased EMPS (Broderick and Reynal, 2009). Non-protein nitrogen is unable to provide the sole source of RDP, and NPN from urea is not as effective as NPN

from true protein (Broderick and Reynal, 2009). For MCP synthesis to be optimized, the RDP in the ration should not solely consist of NPN, but of true protein as well.

Boucher et al. (2007) fed lactating Holstein cows 52% forage diets with 61% of forage from corn silage (DM basis) with increasing amounts of urea (0, 0.3, 0.6, and 0.9% diet DM). As urea concentration increased in the diet, MCP also increased and was maximized at 0.6% urea diet DM (Boucher et al., 2007). Milk yield, milk fat yield and concentration, and protein yield were not affected by supplementation with urea. However, the authors reported a linear increase in MUN concentration and linear decreases in milk protein concentration with increasing amounts of urea in the diet, which resulted in a decrease in milk protein N-to-feed N ratio (Boucher et al., 2007). The increase in MUN concentration was expected because of the increase in CP concentration in the diet. Johnson and Young (2003) reported an inverse relationship between MUN concentration and milk true protein concentration, supporting the results of Boucher et al. (2007). Although urea supplementation increased MCP yield, there were no beneficial effects on milk production, and negative impacts on the environment.

Stanton et al. (2006) listed 6 factors that influence the utilization of urea. The first factor is the source of readily available CHO. When diets are high in digestible energy, the utilization of urea is increased. Rations that are higher in forage usually have a lower utilization of urea and benefit from additional sources of energy. Diets that are high in NFC are better able to handle $NH_3$-N. Also, decreasing the particle size of grain increases ruminal starch digestion and can lead to increased MCP synthesis (Huntington, 1997). Secondly, maintaining a continuous intake of urea improves its utilization compared with

infrequent feedings. This is due to the adaptation that is required for the microbes to produce the enzymes necessary to utilize urea. The dietary concentration of urea fed will also affect its utilization. Low concentrations of urea will be used more efficiently than high concentrations. When urea is thoroughly mixed in with the entire ration, it decreases the chance of the cow consuming a large amount of urea at one time. Instead, the intake of urea will be more consistent, allowing the microbes to use the N from urea for MCP synthesis without creating excess ruminal $NH_3$-N. To increase the utilization of urea, adequate amounts of phosphorus, sulfur, and trace minerals should be available. When urea is substituted for true protein, the diet may be lacking in these minerals, which are necessary for MCP synthesis. Lastly, the solubility of protein affects utilization of urea. True protein sources such as SBM is not as rapidly degraded in the rumen compared with urea, which may make it a better protein supplement in some cases.

**Controlled-Release Urea**

Because of the rising cost of SBM and the risks associated with urea, there have been many developments or modifications made to urea to decrease the risk of toxicity and increase N utilization. Controlled-release urea (**CRU**) products have been developed with the intentions of slowing the release of $NH_3$-N, decreasing the risk of toxicity, improving palatability, and increasing N utilization efficiency in dairy cows (Kertz, 2010). There have been many products developed to slow the release of ammonia from urea. The need for a CRU is to better match the release of the N with the energy source, creating a more synchronized rumen environment and optimizing MCP synthesis. The coating of urea increases processing costs and decreases the N concentration (Table 1) when compared

with urea (Kertz, 2010).

**Table 1.** Concentration of N in different protein supplements (Gehman, 2013)

| N source | % N | % CP[1] |
|---|---|---|
| SRU[2] | 41 | 256 |
| Urea (feed grade) | 45 | 281 |
| Soybean meal | 8 | 50 |

[1]CP = % N × 6.25.
[2]SRU contains 12% ether extract, giving it a lower N concentration than urea.

There have been many advantages by feeing CRU products to lactating dairy cows (Emanuele et al., 2001). For example, supplementation of true protein like SBM can be reduced using CRU. Also, more dietary space in the ration is created for high-quality forages because of the increased N concentration of the supplement. The utilization of low-quality forages can be increased when supplemented with CRU. There is more flexibility when formulating a ration that includes CRU (Tikofsky and Harrison, 2006). Supplementation with CRU decreases the amount of manure produced and the N content of the manure. The increased efficiency of N utilization results in less N excreted into the manure per a unit of milk (Weiss et al., 2007). The rate of ruminal $NH_3$-N release between CRU more closely matched that of SBM which provides the optimum rate of $NH_3$-N for efficient microbial growth (Emanuele et al., 2001). Lastly, ammonia toxicity is decreased, allowing for higher amounts of CRU to be fed compared with urea (Emanuele et al., 2001).

The development of CRU products has been explored for more than 40 years. Some of these include mixtures of gelatinized starch and urea (Bartley and Deyoe, 1975), urea coated in linseed oil (Forero et al., 1980), isobutylidine monourea (Mathison et al., 1994), and biuret (Löest et al., 2001). These products did not perform well, partly because the

NPN was not converted to $NH_3$-N in the rumen, leading to a decrease in MCP synthesis. Although the rate of $NH_3$-N release was slower in the CRU products than urea, it was still too fast for efficient N utilization by the microbes. The results from these products indicate that there is more development and research needed for an improved CRU product.

Alltech (Nicholasville, KY) has developed a CRU product, SRU, which is urea that is coated in vegetable oil. The oil coating is protected in the rumen from microbial attack, slowing the release of N in the urea. The N in SRU is highly concentrated at 256% CP (Tikofsky and Harrison, 2006), but the coating is designed to be inert in the rumen while allowing the release of water-soluble urea through the coating pores, slowing the release of the N (Inostroza, 2009). This allows for the supply of N to the rumen bacteria to be released at a rate that optimizes the synthesis of MCP (Tikofsky and Harrison, 2006).

Inostroza et al. (2010) fed SRU at 114 g/d replacing SBM in 50% forage diets in commercial Wisconsin dairy herds and reported increased milk yield (0.5 kg/d/cow). This is in contrast to Galo et al. (2003) who fed a polymer-coated urea in 50% forage diets consisting of 27.6% corn, 15.7% grass/legume haylage, and 6.8% AH and compared 18% CP diets with urea or polymer-coated urea. The authors reported no differences in milk fat and milk true protein concentrations when supplementing coated urea at 0.77% DM (Galo et al., 2003). dos Santos et al. (2008) also reported no effects on milk yield when SRU was supplemented compared with SBM. The diet with the coated urea excreted more urinary N, suggesting that the N was released more rapidly than expected (Galo et al., 2003). There is a possibility of the increased rate of degradation of the coated urea

products due to damage of the coating that can occur in transportation or mixing of the feed (Galo et al., 2003). Additionally, Galo et al. (2003) found no difference in MCP yield, and the authors concluded that the coated urea product released N faster than expected with lower ruminal starch digestibility, resulting in the lack of effect by supplementing the coated urea product. It is important that the rates of N degradation and CHO fermentation are synchronized to maximize the efficiency of the microbes for MCP synthesis in the rumen.

Effects of supplementing SRU have been inconsistent primarily due to the composition of the diet. Holder (2012) fed angus crossbred steers diets differing in F:C (60:40 vs. 30:70) and NPN source (urea vs. SRU). When steers were fed the higher forage diet, feeding urea resulted in higher body weight (**BW**) gain compared with SRU, but in the diet higher in concentrates, steers fed with SRU increased BW gain over those fed with urea (Holder, 2012). When higher forage diets are fed, there is the chance of limited energy available, which may result in increased ruminal $NH_3$-N concentration. Urea may have resulted in increased ruminal $NH_3$-N concentration compared with SRU. Most cellulolytic bacteria require $NH_3$-N as their source of N (Van Soest, 1994), resulting in improved forage digestibility (Köster et al., 1996) with the increased concentration of ruminal $NH_3$-N. Energy in the 70% concentrate diet would not have been limiting, implying that microbial growth would have been limited more by protein supply. The capture of $NH_3$-N from SRU was probably improved, which may have led to an increase in MCP synthesis, resulting in the improved growth in the higher concentrate diet.

Holder (2012) also indicated when SRU was fed to Holstein steers in high-forage

diets, the rate and extent of ruminal degradation of the SRU was increased compared with a high-concentrate diet. Increased degradation of SRU in high-forage diets may be due to increased activity of microbes that produce urease. High-forage diets typically create a higher ruminal pH and the optimal ruminal pH for urease activity which was reported to be between 6.8 and 8.5 (Mahadevan et al., 1977; Muck, 1982), which increased the degradation rate of SRU.

Figures 3 and 4 depict the in situ patterns of urea disappearance in the rumen for urea and SRU when beef steers were fed a 100% forage or a 70% concentrate diet (Holder, 2012). Urea disappearance was not affected by the F:C and was 100% degraded in the rumen at about 10 min. In contrast, SRU was more degradable in the higher forage diet throughout incubation likely due to higher ruminal pH and fermentative conditions. It took more than 24 h of incubation to release 80 or 90% of urea in SRU for 70% concentrate or 100% forage diet, respectively.

On a dairy, feed costs can account for more than 50% of total costs (Phuong et al., 2013). It generally costs more to feed high producing cows, and so increasing productivity and feed efficiency can improve profitability of the dairy (VandeHaar, 1998). Reducing feed requirements by improving efficiency and decreasing feed costs can be a major contributor for improving dairy profitability (Connor et al., 2012). In a trial conducted by Golombeski et al. (2006) where they fed a CRU product (Ruma-Pro, Unipro International, Greeley, CO) in 50% forage diets consisting of 35% corn silage and 15% AH on a DM basis to lactating cows, the authors reported reduced DMI without affecting milk production, resulting in improved feed efficiency when the CRU was fed.

Using CRU products like SRU has a potential to increase nutrient utilization and efficiency, leading to improved profitability on a dairy.

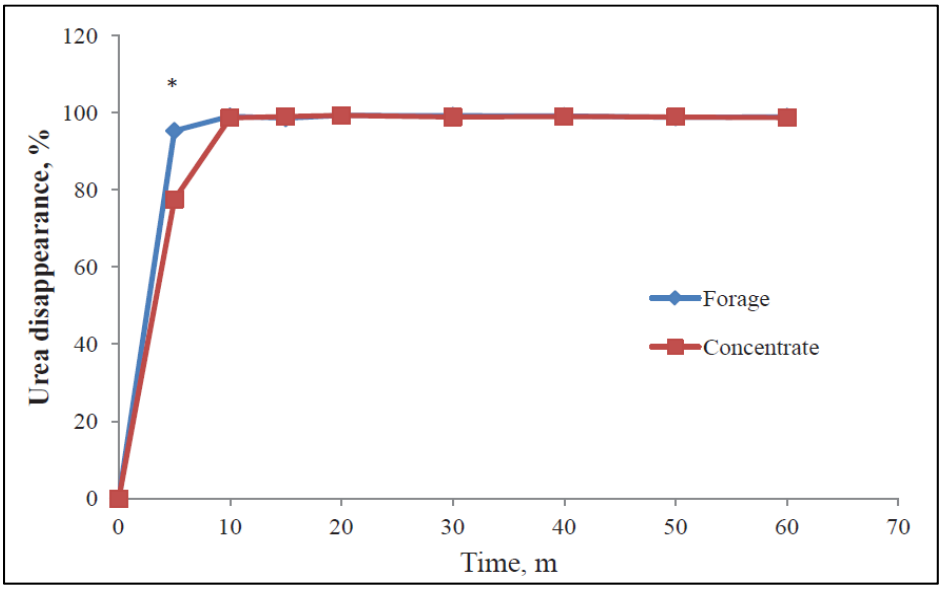


**Figure 3.** Ruminal disappearance of feed grade urea in animals fed 100% forage or 70% concentrate diets (Holder, 2012)
*Treatments differ at indicated time point ($P < 0.05$).

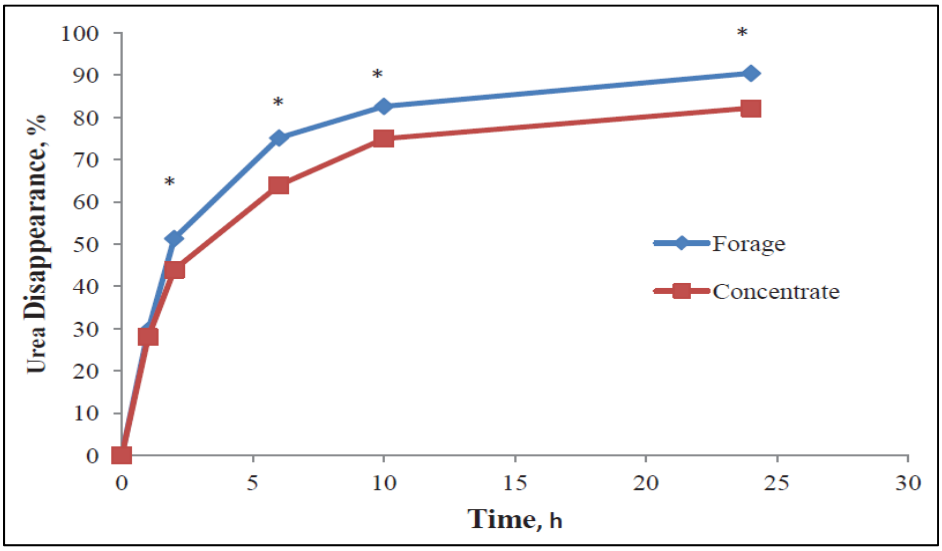


**Figure 4.** Ruminal disappearance of SRU (Alltech, Nicholasville, KY) in animals fed 100% forage or 70% concentrate diets (Holder, 2012)
*Treatments differ at indicated time point ($P < 0.05$).

Several factors should be considered when determining whether substituting a NPN source, such as SRU, is cost-beneficial (Inostroza, 2009). The first consideration to be made is the price of the NPN and the amount that is fed as well as the price of the protein supplement that is going to replace and amount fed. Using NPN in rations increases space for increased DM so that the price of the forage and/or energy supplement that will fill the extra space in the ration must be considered. Lastly, changes in milk yield, milk composition, and the price of milk should be looked at because of incentives that are received based on the quality of milk. Inostroza et al. (2010) performed an economic evaluation of SRU and concluded that IOFC were more favorable when prices of corn grain, corn silage, and SRU were lower, but prices of SBM and milk were higher. The IOFC is calculated by taking the average milk yield of the cow multiplied by the price per kilogram of milk minus the feed cost per cow per day (Inostroza, 2009). Therefore, using IOFC is a good approach to evaluate efficiency and profitability on dairies by feeding protein supplements such as SRU.

**RUP and Ruminally Protected AA**

Increasing the supply of AA to the small intestine without increasing the CP concentration of the diet is becoming more popular with growing concerns of excess N being excreted into the environment. Several technologies have been developed over the years to increase the RUP fraction of protein sources. For example, heat or chemicals have been applied onto oilseed meals, creating ruminally protected AA. Responses to treated oilseed meals and other ruminally protected AA are variable and depend on the method used to protect them from degradation in the rumen (NRC, 2001).

Increasing the RUP in a diet at the expense of RDP to increase AA supply to the small intestine does not always result in positive production responses. Ipharraguerre and Clark (2005b) reported that MCP yield can be decreased when RDP is replaced by high amounts of RUP in the diet. In a review of the effects of RUP on dairy cow performance, Santos et al. (1998) reported that when SBM was replaced by high amounts of RUP in 29 comparisons from 15 metabolism trials, the benefits of increased flow of EAA to the small intestine were not consistent, and decreases in MCP synthesis occurred in 76% of the comparisons in diets supplemented with high amounts of RUP. In the same review, using a different data set with 127 comparisons from 88 lactation studies, the authors found that milk yield increased in only 17% of the comparisons when SBM was replaced by a RUP protein source (Santos et al., 1998). The variability in responses to supplementation with RUP can be explained by the source and concentration of CP in the control diet, the proportion of RUP supplemented in the experimental diet, the effects on MCP synthesis, and the degradability of the RUP (Ipharraguerre and Clark, 2005b).

In early lactation or during negative energy balance, it is even more essential to provide a high quality RUP source in diets. In these situations, limited energy decreases the potential of MCP synthesis, even if there is sufficient RDP. Supplementing RUP during low energy intake periods allows for a high quality source of AA available to the cow that does not need to be converted to MCP first. While this can increase milk production, it also in turn can increase body tissue mobilization (Schei et al., 2005) because of the lack of energy from dietary sources to support the increase in milk production.

Research has shown that at the point of $NH_3$-N overflow in the rumen, adding more RDP will not increase MCP synthesis (Satter and Slyter, 1974), but supplying a high quality AA source can increase milk yield and protein synthesis (Santos et al., 1998; Kalscheur et al., 2006). Dietary escape microbial protein (YMP), developed by Alltech, is a yeast-derived product with similar AA composition to MCP that acts as a RUP and moves with the liquid phase in the rumen. Microbial protein contains 15.8 and 5.2% of total EAA of Lys and Met respectively, while YMP contain 16.0 and 3.6% of total EAA of Lys and Met, respectively (Sabbia et al., 2012). By moving in the liquid phase of the rumen and escaping degradation by ruminal microbes, YMP is able to provide a high quality source of AA to the small intestine to be absorbed for milk production.

Supplementing YMP in lactation diets requires special considerations when formulating rations. For example, NRC (2001) underestimates the passage rate and RUP fraction of YMP, while overestimating its RDP, retention time in the rumen, and MCP yield (Sabbia, 2011). NRC (2001) predicts a passage rate of around 7 %/h for YMP which is under-estimated because YMP has a very similar passage rate to that of the ruminal liquid phase. NRC (2001) divides dietary protein into A, B, and C fractions depending on their ruminal degradability. Fraction A consists of NPN and is instantaneously degraded, when it reaches the rumen (NRC, 2001). Fraction B is the potentially degradable true protein, whereas fraction C is undegradable (NRC, 2001). When YMP was analyzed by in vitro fermentation, the A, B, and C fraction were found to be 20.7, 79.3, and 0, respectively (Sabbia, 2011).

Sabbia et al. (2012) performed a trial using 16 Holstein cows fed high-forage diets

(40% corn silage, 20% AH, and 40% concentrate) replacing SBM with increasing amounts of YMP (0, 1.14, 2.28, and 3.41% DM) to keep diets isonitrogenous at 16.1% CP in a 4 × 4 Latin square design. The authors observed no difference in milk yield, but found a quadratic response in energy-corrected milk because of similar quadratic effects on milk fat concentration and yield (Sabbia et al., 2012). Around 50% of milk fat is synthesized de novo in the mammary gland from precursors including Met (NRC, 2001). The increased concentration of Met from YMP may have allowed for the increase in milk fat synthesis in the mammary gland. Thus, supplementing YMP has a potential as a RUP supplement in high-forage diets or when cows are in a negative energy balance or fed with low-energy diets because of the potential to increase flow of EAA to the small intestine to support milk production.

## Environmental Issues

As the world's population is growing, concerns for protecting the environment are also increasing. To keep up with the increasing demand for food, we as animal scientists need to explore practical ways to increase the production of animals while considering the impacts on the environment. One way to do this is by increasing the production efficiency of the animal. If the animal is better able to utilize the nutrients from less dietary inputs with higher production and lower outputs, we may be able to improve the quality of the environment.

Dairy cows typically produce more ammonia per animal than other livestock due to inefficient utilization of dietary protein in the rumen, leading to significant urinary

excretion with a high concentration of urinary N, particularly when cows are fed with high protein diets. Dairy cattle nutrition needs to establish the balance between feeding minimal amounts of dietary protein required by high producing cows and achieving optimal milk production, while still decreasing negative impacts on the environment. Because excessive N excretion is primarily caused by overfeeding RDP (Rotz, 2004), decreasing the CP concentration in the diet and N intake can decrease total N excretion, including urinary N. Castillo et al. (2001) proposed that excess N intake is primarily excreted into the urine. Dairy cattle rations with 16.5% CP are recommended to support maximum milk and milk protein production, while decreasing N excretion compared with higher CP rations (Colmenero and Broderick, 2006).

Air and water pollution can be caused by ammonia emission from urinary and fecal N found in soil and ground water from dairy farming (Tamminga, 1992). Microbial urease reacts with urinary N (Muck, 1982), and thus urinary N is known to be the most environmentally volatile N (Varel et al., 1999), because urea in urine is rapidly hydrolyzed to ammonia and volatilized into the environment (James et al., 1999). When N is consumed above 500 g/d by cows, about 80% of the N is believed to be excreted into the urine (Castillo et al., 2001). Excess $NH_3$-N in the rumen is absorbed into the blood and transported to the liver where it is converted to urea. Then the urea is either recycled back into the rumen where it can be used again for MCP synthesis or if the needs are already met the urea is excreted into the urine (Bach et al., 2005). Supplementing diets with protein sources like SRU has the potential to decrease urinary N excretion due to lower ruminal $NH_3$-N concentrations by increasing the capture of ruminal $NH_3$-N by

microbes (Galo et al., 2003; Holder, 2012). There are 2 ways N losses can be managed, either by decreasing protein degradation or increasing the N use by the rumen microbes (Bach et al., 2005).

One way to decrease negative impacts of N on the environment is to increase the efficiency of protein utilization in the cow, which results in less N excreted per unit of milk produced (Weiss et al., 2007). Increased nutrient utilization can lead to increased profitability on the farm as well as less excreted waste (Holder, 2012). Jonker et al. (2002) reported that N utilization efficiency was decreased by 0.05 percentage units for every additional gram of N in the diet over the recommended intake of N. Optimal supply of RDP and efficiency of utilization of absorbed AA for milk protein synthesis should be the main focus when looking to improving N utilization efficiency (Dijkstra et al., 2013). When dietary protein is in excess, N utilization efficiency decreases, leading to greater amounts of N losses into urine and feces (Tamminga, 1992).

There is a body of evidence to indicate that milk N efficiency is decreased, when N intake is increased (Castillo et al., 2000; Kalscheur et al., 2006; Dijkstra et al., 2013). Castillo et al. (2000) reported that up to 400g N intake/d, there is a positive relationship between N intake and milk N, but when N intake is above 400 g N intake/d, there is a negative relationship. Kalscheur et al. (2006) fed cows diets consisting of 50% corn silage and 50% concentrate on a DM basis with 4 different concentrations of RDP (6.8, 8.2, 9.6, and 11.0% DM) with the same RUP concentration. Dry matter intake was not different between treatments, but there was a liner increase in CP intake and a linear decrease in N utilization efficiency, as RDP concentration was increased in the diet

(Kalscheur et al., 2006). Figure 5 indicates that although N intake increased milk N output, it was not related to milk N efficiency. However, N concentration in the diet was related to milk N efficiency, signifying the importance of feed intake on N efficiency (Dijkstra et al., 2013).

Because ruminants are able to convert resources that humans cannot or choose not to consume into high-quality food products, they play an important role in human food production. However, the efficiency of converting these resources into food is not always the most efficient and causes unavoidable losses of N into the environment. Improving N utilization efficiency in ruminants, especially dairy cattle, can improve environmental sustainability by decreasing urinary N output, reducing nitrate leaching and ammonia volatilization, and mitigating nitrous oxide emissions, which will reduce the detrimental impacts on the environment and contribute to sustainable dairy production.

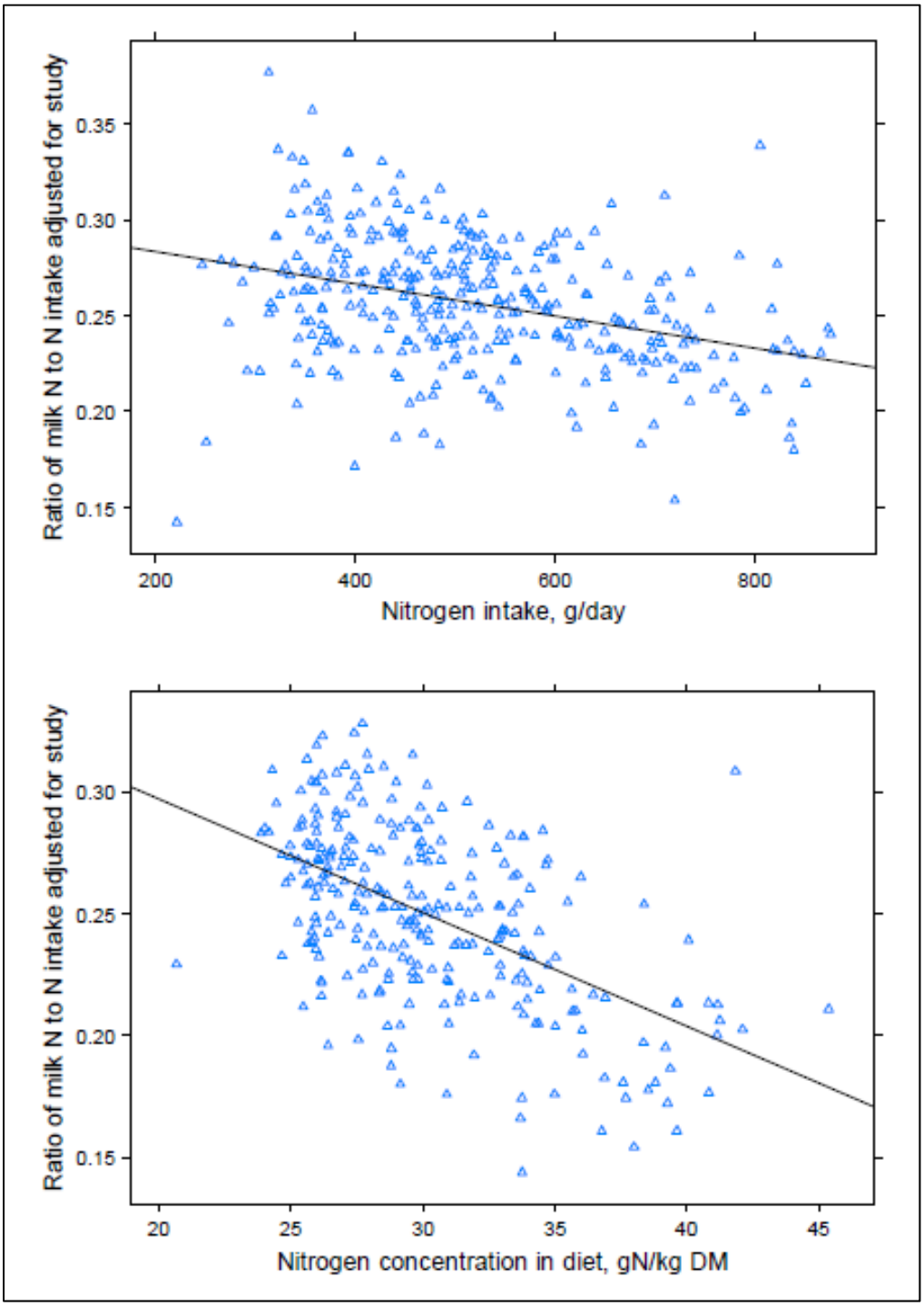


**Figure 5.** Relationship between N intake and efficiency of milk N-to-N intake and the relationship between N concentration of the diet and efficiency of milk N-to-N intake (Dijkstra et al., 2013)

# MATERIALS AND METHODS

The dairy cows used in this study were cared for according to the Live Animal Use in Research Guidelines of the Institutional Animal Care and Use Committee at Utah State University. The study was conducted at the Caine Dairy Research Center (Wellsville, UT), Utah State University from October 18, 2012 to April 15, 2013.

## Cows, Experimental Design, and Diets

Twelve multiparious lactating Holstein cows, 4 of which were surgically fitted with rumen cannula, were used. Cows began the experiment averaging 46 ± 8.1 DIM. Average BW were 717 ± 48.9 and 730 ± 43.2 kg at the beginning and the end of the experiment, respectively.

A triple 4 × 4 Latin square design was used with one square comprised of ruminally cannulated cows. The experiment consisted of 4 periods lasting 28 d each (21 d of treatment adaptation and 7 d of data and sample collection). Within each square, cows were randomly assigned to a sequence of 4 dietary treatments without or with added protein supplements: 1) no supplement as a control, 2) a total mixed ration (**TMR**) containing SRU (**SRUT**), 3) a TMR containing YMP (**YMPT**), 4) or a TMR containing SRU and YMP (**SYT**). The SRU was supplemented at 0.49% DM in the SRUT and the SYT in order for cows to consume approximately 127 g/d. The dietary concentration of the SRU was chosen based on a previous lactation study (Inostroza et al., 2010). The YMP was added at 1.15% DM in the YMPT and the SYT treatments in order for cows to consume approximately 299 g/d (Sabbia et al., 2012). Isonitrogenous condition between

treatments was maintained by replacing mixture of SBM and canola meal (**SBMCM**) in 50:50 with the SRU and/or the YMP (Table 2). In addition, diets had similar RDP and RUP fractions.

Diets were formulated based on the NRC (2001) recommendations to provide sufficient net energy for lactation ($\mathbf{NE_L}$), metabolizable protein, vitamins, and minerals to produce 40 kg/d of milk with 3.5% fat and 3.0% true protein (**TP**). Diets were isonitrogenous and isocaloric ($NE_L$ basis) averaging 16.0% CP and 1.66 Mcal/kg DM, respectively (Table 2). The AH used in our study had a chemical composition of 20.3, 36.0, and 25.9% DM for CP, NDF, and acid detergent fiber (**ADF**) respectively, whereas corn silage had a chemical composition of 7.36, 39.0, and 21.1% DM for CP, NDF, and ADF, respectively.

Cows were housed individually in tie stalls fitted with rubber mattresses covered with straw, allowing free access to water. Cows were individually fed twice daily for ad libitum intake at a level of 110% expected daily intake with 70% of allotted feed fed at 0600 h and 30% fed at 1500 h. Feed offered and refused was recorded daily, and samples taken during the sampling week to determine DMI.

Cows were milked twice daily at 0400 and 1600 h, and milk production was recorded throughout the entire experiment. Milk was sampled for 2 consecutive d (d 22 and d 23) during the a.m. and p.m. milkings each period. Milk samples were stored at 4°C and preserved with Broad Spectrum Microtabs II (D & F Control Systems Inc., San Ramon, CA). Individual milk samples were analyzed by the Rocky Mountain DHIA Laboratory (Wellsville, UT) for fat, TP, lactose, and MUN. Milk composition was expressed on

weighted milk yield of a.m. and p.m. samples. Milk fat and TP yields were calculated by multiplying milk yield from the respective day by fat and TP concentration of the milk on an individual cow. To convert milk TP to milk N, 6.38 was used as the conversion factor (DePeters and Cant, 1992), and total milk N (kg/d) was calculated as milk TP/6.38 + MUN, where milk TP and MUN were expressed as kg/d.

Cows were weighed for 2 consecutive d after the a.m. milking and before the a.m feeding at the beginning and end of each period. These weights were used to calculate the mean BW of cows for each experimental period. Energy utilization was determined by calculating energy for maintenance as $BW^{0.75}\times$ 0.08 (NRC, 2001). Energy of BW change was assumed to be 5.114 Mcal/kg of gain or 4.924 Mcal/kg of loss (NRC, 2001). Milk energy was calculated as (0.0929 × milk fat concentration) + (0.0563 × milk TP concentration) + (0.0395 × milk lactose concentration) (NRC, 2001). Intake of $NE_L$ was estimated from DMI and estimated $NE_L$ contents (NRC, 2001).

## Feed Sampling and Analysis

Samples of AH and corn silage were taken weekly to determine DM, and diets were adjusted accordingly for change in DM concentration. Samples were composited by month, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Swedesboro, NJ), and stored for chemical analysis. Samples of TMR and orts were collected from individual cows on d 22 to d 28, composited, dried at 60°C for 48 h, and ground as previously described. The DM concentrations of samples were used to calculate intakes of DM and nutrients.

**Table 2.** Ingredient and chemical composition of the experimental diets without or with protein supplements fed to lactating Holstein dairy cows (n = 4)

| | Experimental diets[1] | | | | | |
|---|---|---|---|---|---|---|
| Item | Control | SRUT | YMPT | SYT | SEM | *P* |
| Ingredient, % DM | | | | | | |
| Alfalfa hay | 24.5 | 24.5 | 24.5 | 24.5 | - | - |
| Corn silage | 28.0 | 30.4 | 28.0 | 30.4 | - | - |
| Corn grain, steam-flaked | 21.0 | 21.0 | 21.0 | 21.0 | - | - |
| Corn DDGS[2] | 7.87 | 7.87 | 7.87 | 7.87 | - | - |
| Cottonseed, whole | 5.24 | 5.24 | 5.24 | 5.24 | - | - |
| Beet pulp, shreds | 5.24 | 5.24 | 5.24 | 5.24 | - | - |
| SBMCM[3] | 5.59 | 2.62 | 4.44 | 1.47 | - | - |
| Optigen | - | 0.49 | - | 0.49 | - | - |
| DEMP | - | - | 1.15 | 1.15 | - | - |
| Sodium bicarbonate | 0.52 | 0.52 | 0.52 | 0.52 | - | - |
| Vitamin and mineral mix[4] | 2.10 | 2.10 | 2.10 | 2.10 | - | - |
| Chemical composition, % DM | | | | | | |
| DM, % | 58.3 | 55.4 | 58.9 | 56.7 | 1.27 | 0.23 |
| OM | 91.2 | 91.3 | 91.6 | 91.2 | 0.23 | 0.61 |
| CP | 16.3 | 15.5 | 16.2 | 16.0 | 0.52 | 0.72 |
| RDP[5] | 8.49 | 8.61 | 8.42 | 8.55 | - | - |
| RUP[5] | 7.16 | 7.12 | 7.21 | 7.15 | - | - |
| NDF | 32.2 | 32.1 | 31.7 | 32.9 | 0.67 | 0.67 |
| ADF | 18.6 | 18.6 | 18.0 | 19.1 | 0.49 | 0.52 |
| Ether extract | 2.86[b] | 3.48[a] | 3.85[a] | 3.73[a] | 0.150 | < 0.01 |
| NFC[6] | 39.7 | 40.2 | 40.0 | 38.6 | 0.98 | 0.67 |
| $NE_L$,[5] Mcal/kg | 1.67 | 1.65 | 1.67 | 1.65 | - | - |

[a-b]Means within a row that do not have a common superscript differ at $P < 0.05$.

[1]Control = TMR without protein supplement; SRUT = TMR with slow released urea (Optigen, Alltech Inc., Nicholasville, KY); YMPT = TMR with yeast-derived microbial protein (DEMP, Alltech Inc.); and SYT = TMR with slow released urea and yeast-derived microbial protein.

[2]DDGS = dried distillers grains with solubles.

[3]Mixture of soybean meal and canola meal at 50:50 in a DM basis.

[4]Formulated to contain (per kg DM): 226.7 mg of Se (from sodium selenite), 9278.7 mg of Cu (from copper amino acid complex), 40,537.4 mg of Zn (from zinc amino acid complex), 38,653.4 mg of Mn (from manganese amino acid complex), 552.6 mg of Co (from cobalt carbonate), 1,234,585.2 IU of vitamin A, 152,808.1 IU of vitamin D, 3,815.1 IU of vitamin E, and 295 mg of Rumensin (Elanco Animal Health, Greenfield, IN).

[5]Based on tabular value (NRC, 2001)

[6]NFC = 100 – CP – NDF – ether extract – ash.

Analytical DM concentration of samples was determined by oven drying overnight at 105°C, and organic matter (**OM**) was determined by ashing at 550°C for 5 h (AOAC, 2000; method 942.05). Concentration of CP was determined using an automated N combustion analyzer (Elementar, Analysensysteme GmbH, Hanau, Germany; AOAC, 2000; method 968.06). Concentrations of NDF and ADF were sequentially determined using a fiber analyzer (200/220, ANKOM Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination and pre-treated with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO). Ether extract was measured using a fat analyzer (XT20, ANKOM Technology; AOAC, 2000; method 2003.05).

## Ruminal Fermentation Characteristics

Ruminal pH was measured continuously starting on d 24 for 2 consecutive d using indwelling pH meters in the cannulated cows. The Lethbridge Research Centre Ruminal pH Measurement System (**LRCpH**; Dascor, Escondido, CA) as described by Penner et al. (2006) was used. Prior to placing the LRCpH system in the rumen, readings in pH buffers 4 and 7 were recorded. Meters were placed in the rumen taking a pH measurement every 30 s, which was stored by the data logger. The LRCpH was removed from the rumen after 48 h of continuous pH measurements and washed in 39°C water. The daily ruminal pH data were averaged for each minute and summarized as minimum, mean, and maximum pH. Also, when ruminal pH was less than 5.8, daily episodes,

duration (h/d), and area (pH × min) were calculated. The threshold of 5.8 was chosen because it has been previously described by others (Beauchemin and Yang, 2005) to cause ruminal acidosis.

Ruminal contents were sampled from cannulated cows at 0, 3, and 6 h after the a.m. feeding on d 26 and d 27. Approximately 1 L of ruminal contents was obtained from different locations within the rumen (anterior dorsal, anterior ventral, medial ventral, posterior dorsal, and posterior ventral) and strained through a polyester screen (pore size 355 μm; B & S H Thompson, Ville Mont-Royal, QC, Canada). Five mL of the filtered ruminal fluid was added to 1 mL of 1% sulfuric acid, and samples were retained for $NH_3$-N determination. Concentration of $NH_3$-N in the ruminal contents was measured as described by Rhine et al. (1998). Another 5 mL of filtered ruminal fluid was added to 1 mL of 25% meta-phosphoric acid, and the samples were retained for VFA determination. The VFA were quantified using a gas chromatograph (model 5890, Hewlett-Packard Lab, Palo Alto, CA) with a capillary column (30 m × 0.32 mm i.d., 1 μm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame-ionization detection. The oven temperature was 170°C held for 4 min, which was then increased by 5°C/min to 185°C, and then by 3°C/min to 220°C, and held at this temperature for 1 min. The injector temperature was 225°C, the detector temperature was 250°C, and the carrier gas was helium (Eun and Beauchemin, 2007).

## Urine Sampling and Analysis for MCP Production

On d 22 to d 24 spot urine samples were collected from each cow at 0600 and 1800 h

for a total of 6 samples per cow (Holt et al., 2013). Using 4 *M* HCl urine samples were acidified to pH < 4.0 and composited by cow per period. Samples were frozen and stored at –40°C. Samples were thawed at a later date for analysis and diluted with 39 parts diluent to 1 part urine. Diluent consisted of 0.202% sodium 1-heptane sulfonic acid and 0.086% ammonium dihydrogen phosphate ($NH_4H_2PO_4$), and the solution was brought to a pH of 2.1 using 4 *M* HCl. Using the ratio of urinary purine derivatives (**PD**) to creatinine is an accepted way to estimate the MCP flow to the duodenum (Shingfield and Offer, 1998). The PD and creatinine were analyzed using an HPLC instrument (Waters Corp., Milford, MA) according to the procedures of Shingfield and Offer (1999). Creatinine is used as a marker to estimate urine volume (Valadares et al., 1999). An average creatinine output value of 28 mg/kg of BW estimated by Whittet (2004) was used in calculating urine volume. Others have also reported similar creatinine outputs, ranging from 25 to 30 mg/kg of BW daily (Jones et al., 1990). To estimate the relative differences in MCP production, the ratio of urinary PD (allantoin and uric acid) to creatinine was used (Shingfield and Offer, 1998). Supply of MCP was estimated based on estimates of urinary excretion of PD according to the method of Chen and Gomes (1992).

## Calculation of IOFC

Milk and its component prices used to calculate IOFC were averaged for the entire trial from the months of October, 2012 to April, 2013. The IOFC were calculated using the average blend price per hundredweight (**cwt**) basis of milk for the local milk manufacturing company and component prices from the Pacific Northwest (Federal

Order No. 124; USDA-Agricultural Marketing Service, Dairy Programs, Phoenix, AZ). Feed prices were reflective of local prices. The average prices used were $18.95, 4.00, 7.18, and 0.97 for price/cwt milk, kg fat, kg protein, and kg other solids, respectively. Lactose was used in place of other solids in the component pricing formula, because we did not have a value for other solids and assumed that the differences due to minerals were consistent across treatments. Varying IOFC were calculated based on milk yield, ECM yield, and the value of milk based on the value of components compared with actual DMI.

## Statistical Analysis

Data were summarized for each cow by measurement period. All data were analyzed with a model that included the fixed effect of dietary treatment using the repeated option in the mixed model procedure of SAS (SAS Institute, 2012). Cow and period were the terms of the random statement. The relationship between N intake and efficiency of use of feed N to milk N was determined by linear regression using the PROC REG procedure of SAS. Simple, autoregressive one, and compound symmetry covariance structures were used in the analysis depending on low values for the Akaike's information criteria and Schwartz's Bayesian criterion. Data for intakes of DM and nutrients, VFA, and N utilization were reported using the variance components structure, whereas milk yield was analyzed by the unstructured covariance structure. Data for milk components and efficiency were analyzed using the compound symmetry covariance structure. In addition, data for $NH_3$-N and MCP yield were analyzed by the heterogeneous compound

symmetry structure.

For all models used, degrees of freedom were estimated with the Kenward-Roger specification in the models. Means were compared using a protected ($P < 0.10$) LSD test. Least square means are reported throughout. Treatment effects were declared significant at $P < 0.05$, and differences were considered to indicate a trend toward significance at $0.05 < P < 0.10$.

# RESULTS AND DISCUSSION

A large-scale field trial in Wisconsin commercial dairy herds indicated that SRU supplementation was an effective partial substitute for SBM in high-forage diets by increasing milk yield and IOFC (Inostroza et al., 2010). Another lactation dairy experiment showed that substitution of SBM with YMP tended ($P < 0.09$) to improve 4.0% FCM and ECM yields by dairy cows consuming high-forage diets (Sabbia et al., 2012). The positive results reported in these previous studies led us to conduct the present experiment to investigate nutrient metabolism and utilization of dairy cows fed high-forage diets based on AH and its impacts on IOFC by supplementing with SRU and YMP.

## Diet Composition and Dietary Treatments

Ingredients and chemical composition of experimental diets are listed in Table 2. All diets contained a high proportion of forages consisting on average of 53.7% of TMR as forage DM with 45.7% of forage DM from AH. On a RDP basis, 7.1 g of SBM is equivalent to 1 g of SRU (Tikofsky and Harrison, 2006), and consequently the N in SRU is more concentrated compared with SBMCM, allowing more dietary space for DM in the diet. Treatments SRUT and SYT had 2.4% more DM from corn silage than the control and YMPT. To maintain an isonitrogenous condition between treatments, the SBMCM was added in decreasing concentrations, as SRU, YMP, or their combination was added to the diets. Although concentration of ether extract differed between the control and protein-supplemented diets, NFC concentration was similar across

experimental diets averaging 39.6% DM.

## Intake and Milk Production

Intakes of DM and nutrients decreased when protein supplements were added to the diet (Table 3). Intakes of NDF and ADF were especially decreased in YMPT compared with the control because of the lower DMI in YMPT. Intake of DM as a proportion of BW of cows tended to decrease ($P = 0.10$) with adding protein supplements. The negative impact of protein supplements on DMI observed in this study was unexpected, and the mechanism whereby protein supplements decreased DMI is difficult to explain. Sabbia et al. (2012) fed diets with increasing amounts of YMP, while decreasing amounts of SBM in high-forage diets consisting of 60.5% forage DM with 41.6% DM from corn silage and 18.9% DM from AH. In the study, cows fed with 0, 1.14, 2.28, and 3.41% DM of YMP showed a cubic effect on DMI (Sabbia et al., 2012). In contrast to our findings, they observed an increase in DMI in cows fed with 1.14% DM (300 g/d) of YMP compared with a control (0% YMP; Sabbia et al., 2012). It is known that urea can be fed to lactating dairy cows up to a concentration of 1% of the total ration without negative impacts on DMI (Kertz, 2010). In the current study, SRU was included at a rate of 0.49% DM in SRUT and SYT, suggesting that supplementing SRU at this rate would not result in the decreased DMI. Akay et al. (2004) reported a decrease of 0.90 kg/d in DMI when a similar product to SRU was fed to cows replacing SBM, urea, and whole cottonseed in a control diet, while still maintaining similar milk yield. Allen (2000) reported that DMI will continue to increase until gut fill is no longer a limiting factor and then decrease with

**Table 3.** Productive performance, BW change, and net energy utilization of lactating Holstein dairy cows fed alfalfa hay-based TMR without or with protein supplements

| | Dietary treatments[1] | | | | | |
|---|---|---|---|---|---|---|
| Item | Control | SRUT | YMPT | SYT | SEM | *P* |
| Intake, kg/d | | | | | | |
| DM | 29.4[a] | 27.9[ab] | 26.5[b] | 27.5[b] | 0.84 | 0.02 |
| DM, % of BW | 3.99 | 3.76 | 3.55 | 3.71 | 0.143 | 0.10 |
| OM | 26.8[a] | 25.5[ab] | 24.3[b] | 25.1[b] | 0.79 | 0.03 |
| CP | 5.00[a] | 4.48[b] | 4.23[b] | 4.46[b] | 0.179 | 0.01 |
| NDF | 9.42[a] | 8.76[bc] | 8.40[c] | 9.09[ab] | 0.276 | < 0.01 |
| ADF | 5.47[a] | 5.00[bc] | 4.75[c] | 5.29[ab] | 0.180 | < 0.01 |
| Yield, kg/d | | | | | | |
| Milk | 39.7 | 40.7 | 41.1 | 40.2 | 1.97 | 0.08 |
| 3.5% FCM | 38.8 | 41.5 | 42.7 | 41.0 | 2.43 | 0.08 |
| ECM | 39.5 | 41.7 | 43.0 | 41.2 | 2.26 | 0.07 |
| Milk composition, % | | | | | | |
| Fat | 3.43 | 3.64 | 3.70 | 3.64 | 0.152 | 0.39 |
| True protein | 3.05 | 3.04 | 3.08 | 3.01 | 0.076 | 0.41 |
| Lactose | 4.90 | 4.90 | 4.90 | 4.91 | 0.058 | 0.99 |
| Milk component yield, kg/d | | | | | | |
| Fat | 1.34 | 1.49 | 1.53 | 1.46 | 0.098 | 0.10 |
| True protein | 1.19 | 1.21 | 1.27 | 1.20 | 0.051 | 0.07 |
| Lactose | 1.95 | 2.00 | 2.05 | 1.97 | 0.119 | 0.25 |
| Efficiency | | | | | | |
| Milk yield/DMI | 1.35[b] | 1.46[a] | 1.55[a] | 1.46[a] | 0.092 | 0.04 |
| 3.5% FCM yield/DMI | 1.32[b] | 1.49[a] | 1.61[a] | 1.49[a] | 0.091 | 0.03 |
| ECM yield/DMI | 1.34[b] | 1.49[a] | 1.61[a] | 1.50[a] | 0.085 | 0.01 |
| BW | | | | | | |
| Initial, kg | 708 | 716 | 722 | 721 | 14.7 | 0.08 |
| Mean, kg | 733 | 727 | 733 | 727 | 14.9 | 0.62 |
| Change, kg/d | 0.76[a] | 0.27[b] | 0.27[b] | 0.08[b] | 0.174 | 0.04 |
| Net energy utilization, Mcal/d | | | | | | |
| Maintenance | 11.3 | 11.2 | 11.3 | 11.2 | 0.17 | 0.64 |
| BW change | 3.97[a] | 1.41[b] | 1.36[b] | 0.40[b] | 0.883 | 0.04 |
| Milk | 27.4 | 28.3 | 30.0 | 28.0 | 1.39 | 0.08 |
| Total[2] | 42.7 | 41.0 | 42.7 | 39.6 | 1.56 | 0.17 |
| $NE_L$, Mcal/kg DMI | 1.48 | 1.53 | 1.59 | 1.45 | 0.06 | 0.18 |

[a-b]Means within a row that do not have a common superscript differ at $P < 0.05$.

[1]Control = TMR without protein supplement; SRUT = TMR with slow released urea (Optigen, Alltech Inc., Nicholasville, KY); YMPT = TMR with yeast-derived microbial protein (DEMP, Alltech Inc.); and SYT = TMR with slow released urea and yeast-derived microbial protein.

[2]Net energy used for maintenance, BW change, and milk.

an excess of metabolic fuels. Cows receiving protein supplements in this study may have received all necessary nutrients from lower feed intakes, decreasing the DMI. Another possibility is that shifts in energy metabolism due to adding protein supplements may have contributed to the downward regulation of intakes of cows fed protein supplements. Further research needs to be done to determine the mechanism of regulation of feed intake when high-forage diets are supplemented with SRU and/or YMP.

Cows fed with protein supplements tended to increase yields of milk ($P = 0.08$), 3.5% FCM ($P = 0.08$), and ECM ($P = 0.07$) compared to those fed the control (Table 3). Ipharraguerre (2004) found that replacing RDP with RUP increased milk yield by 2.1% when diets ranged from 16.0 to 17.9% CP. The greatest response in milk yield due to feeding YMPT suggests that there was enough RDP from SBMCM and AH to maintain MCP synthesis as well as an increasing supply of Lys and Met absorbed in the small intestine from YMP to support the increase in milk production. Milk fat, TP, and lactose concentrations averaged 3.60, 3.05, and 4.90%, respectively, and did not differ between treatments. Although there were no differences in milk fat and TP concentrations, due to a tendency ($P = 0.08$) for the increase in milk yield and numerical increases in milk fat and TP concentrations with feeding YMPT, cows fed YMPT tended to increase milk fat ($P = 0.10$) and TP yields ($P = 0.07$). Akay et al. (2004) reported a decreased milk protein concentration, but an increase in milk yield of 3.7 kg/d, resulting in an increased milk protein yield when a similar SRU product to the one tested in this study was supplemented in diets containing 47.5% forage and 58.5% concentrate on a DM basis. The authors concluded that the response in milk yield was due to improved MCP

synthesis and increased ruminal starch digestion (Akay et al., 2004). Contrary to our results, Inostroza et al. (2010) reported a tendency for milk fat concentration to decrease ($P = 0.07$) when SRU was supplemented compared with the control in high-forage diets. Increasing YMP supplementation tended ($P = 0.06$) to have a quadratic effect on milk fat concentration in high-forage diets containing SBM as the main dietary protein source, resulting in YMP added at 1.14% DM to have an increased milk fat concentration (3.66%) compared with a control (3.53%; Sabbia et al., 2012). Around 50% of milk fat is synthesized de novo in the mammary gland from varying precursors including dietary Met. It is still unclear why AA affect milk fat synthesis, but it may be due to the effect that Met has on the synthesis of short- and medium-chain fatty acids (NRC, 2001). Also, AA may have a role in hepatic synthesis of chylomicrons and very-low density lipoproteins (NRC, 2001; Guretzky et al., 2006). In addition, when Met supply is limited, synthesis of phospholipids can be affected because of Met requirement in choline synthesis and choline's contribution to the formation of phospholipids (NRC, 2001). Methionine acts as a methyl donor for choline synthesis, and consequently insufficient Met supply may lead to decreased milk and milk fat yields (NRC, 2001; Guretzky et al., 2006). Concentrations of Lys and Met as a percentage of total essential AA in YMP averaged 16.0 and 3.6% (Sabbia et al., 2012), respectively, whereas ruminal MCP averaged 15.8 and 5.2% for Lys and Met, respectively (Clark et al., 1992; NRC, 2001). It is well accepted that for a RUP supplement to be effective its AA composition should complement the AA profile of MCP (Santos et al., 1998). Under negative energy balance, dairy cows are typically unable to eat enough to meet their energy and protein

requirements, forcing them to mobilize body fat and making it a more critical time for AA supplementation (Schei et al., 2005). Cows fed YMPT ate less, but had more AA available for absorption, suggesting that more Met and Lys from YMP may have been able to escape ruminal fermentation and be absorbed in the small intestine, resulting in the tendencies for increases in yields of milk, milk fat, and TP.

## Feed Efficiency, BW, and Net Energy Utilization

Feed efficiencies based on yields of milk, 3.5% FCM, and ECM increased when treatments were supplemented with SRU and/or YMP (Table 3). There was no difference in mean BW between treatments, but feeding protein supplements caused less BW gain compared with the control (Table 3). Net energy for maintenance did not differ between treatments averaging 11.2 Mcal/d, whereas net energy utilized for BW gain was greater in the control (3.97 Mcal/d) compared with protein-supplemented treatments (1.06 Mcal/d on average). Feeding YMPT tended ($P = 0.08$) to have the greatest net energy utilized for milk production. Total $NE_L$ was not different between treatments. Although experimental diets tested in the current study were formulated to contain 1.66 Mcal/kg of $NE_L$ on average, differences in DMI and yields of milk and its components resulted in lower $NE_L$ values (1.51 Mcal/kg DMI).

Feed efficiencies are used to evaluate herd productivity and profitability, and increased feed efficiencies are associated with greater milk yield, loss in body condition, high-quality forages, and improved feed digestibilities (Britt et al., 2003). Protein-supplemented treatments tended ($P = 0.08$) to increase milk yields and had less net

energy utilized for BW gain, along with a decrease in DMI compared with the control (Table 3). Vallimont et al. (2011) found that BW and body condition score were negatively but strongly correlated with feed efficiency ($R^2$ = –0.64 and –0.70, respectively). Cows that lose more BW during lactation are more efficient, but when measuring feed efficiency, the difference between energy from dietary inputs and body tissue mobilization are not differentiated (Connor et al., 2012). This allows for cows that mobilize more body tissue, to have greater feed efficiency. Cows fed with SRU and/or YMP showed less net energy utilized for BW gain compared with the control (Table 3), suggesting that the cows fed with the protein supplements may have mobilized more body tissue to support milk production, resulting in increased feed efficiency. Although SRU and/or YMP supplementation resulted in similar increases in the feed efficiencies, feeding YMPT caused the greatest responses among protein-supplemented diets.

Noteworthy is that an interaction between source of main forage in lactation diets and stage of lactation can affect feed efficiency when supplementing RUP such as YMP (Wattiaux and Karg, 2004). For instance, Sabbia et al. (2012) reported no differences in ECM/DMI between a control diet and a diet supplemented with 1.14% YMP. In that study, cows in mid- to late lactation fed with 1.14% YMP increased BW compared to those fed a control (Sabbia et al., 2012), implying that more dietary energy was used for BW gain instead of milk production, causing no response in feed efficiency. The lack of effect on feed efficiency due to the YMP supplementation may have been caused by diet composition and stage of lactation. Sabbia et al. (2012) fed cows with a higher proportion of corn silage (41.6%) than AH (18.9%), which may have provided more readily

fermentable energy from the diet, decreasing the need for body tissue mobilization to support milk production. Additionally, cows in mid- to late lactation do not extensively mobilize body tissue energy to support their milk production. In these conditions, protein supplements may have limited effects on nutrient utilization and feed efficiency, which suggests that YMP supplementation may have a better response in cows fed with a relatively low concentration of corn silage and when they are in early lactation or under negative energy balance.

The shift in net energy utilization with decreased DMI due to protein supplementation observed in this study suggests that adding protein supplements channeled more absorbed energy substrates and nutrients into the mammary gland, but minimized them to BW gain. In early lactation or low metabolizable energy (**ME)** intake situations, a greater supply of RUP can be provided to the cow to meet its protein requirement. While this can increase milk production, it also in turn can increase body tissue mobilization (Schei et al., 2005). More RUP may have been supplied in YMPT, which may account for the decrease in BW change compared with the control. Feeding protein-supplemented diets in this study increased mobilization of body tissue to support milk production, which resulted in increased feed efficiency. It has been suggested that when energy is available with limited AA supply, energy utilization efficiency is decreased for milk production, and more energy is partitioned toward body tissue (Huhtanen, 1998). The control diet may have had enough energy for milk production, but been limited in its AA supply compared with YMPT, causing the decrease in milk production and the increase in net energy utilized for BW.

## Ruminal Fermentation Characteristics

Feeding SYT tended to decrease mean and maximum pH in the rumen ($P = 0.06$; Table 4); however, mean pH from all dietary treatments were at least above 5.80, which was expected, as we fed high-forage diets. An in vitro study performed by Sabbia et al. (2012) resulted in no differences in ruminal pH (averaging 6.46) when increasing amounts of YMP replaced SBM. The authors observed similar results in vivo, with ruminal pH averaging 6.42 when cows were fed the same treatments tested in vitro (Sabbia et al., 2012). No responses in ruminal pH were detected when rumen-simulating fermentors were offered 50% forage diets consisting of 25% corn silage and 25% AH (DM basis) with either urea or SRU supplementation (Tikofsky and Harrison, 2006). These results indicate that there would be minimal effects on physiological conditions of ruminal microbial fermentation when SRU and/or YMP are supplemented in high-forage diets.

Cows fed protein-supplemented diets tended to decrease total VFA concentration ($P = 0.07$) compared to those fed the control (Table 4). Feeding SRUT decreased molar proportion of propionate, which contributed to a tendency to increase (acetate+butyrate):propionate ($P = 0.08$). Holder et al. (2012) also reported decreased propionate proportion when growing Holstein steers were fed with SRU at 12.1% CP. Sabbia et al. (2012) reported no differences in total VFA concentration and individual VFA proportions, except for decreased isovalerate when increasing amounts of YMP were supplemented in high-forage diets. Fiber digesting bacteria in the rumen have a preference for $NH_3$-N while producing more acetate than propionate (Van Soest, 1994). It

is possible that when SRU is added to high-forage diets, the release of $NH_3$-N from SRU provides the fiber digesting bacteria with the N they need, producing more acetate but less propionate, which may increase the (acetate+butyrate):propionate.

**Table 4.** Ruminal fermentation characteristics of lactating Holstein dairy cows fed alfalfa hay-based TMR without or with protein supplements

| | Dietary treatments[1] | | | | | |
|---|---|---|---|---|---|---|
| Item | Control | SRUT | YMPT | SYT | SEM | *P* |
| Minimum pH | 5.54 | 5.55 | 5.51 | 5.41 | 0.083 | 0.21 |
| Mean pH | 6.27 | 6.34 | 6.25 | 6.09 | 0.066 | 0.06 |
| Maximum pH | 7.24 | 7.18 | 7.02 | 6.87 | 0.109 | 0.06 |
| pH < 5.8 | | | | | | |
| Daily episodes | 23.8 | 15.5 | 17.0 | 20.3 | 10.34 | 0.92 |
| Duration, h/d | 1.45 | 3.27 | 3.32 | 8.70 | 2.475 | 0.17 |
| Area, pH × min | 10.7 | 35.0 | 31.8 | 34.8 | 16.64 | 0.46 |
| Total VFA, m*M* | 140 | 134 | 127 | 131 | 4.4 | 0.07 |
| Individual VFA[2] | | | | | | |
| Acetate (A) | 59.3 | 60.6 | 59.7 | 59.2 | 1.20 | 0.22 |
| Propionate (P) | 25.5[a] | 24.1[b] | 25.3[ab] | 26.0[a] | 1.43 | 0.05 |
| Butyrate (B) | 11.2 | 11.5 | 11.1 | 10.8 | 0.34 | 0.38 |
| Valerate | 1.66[a] | 1.46[b] | 1.51[b] | 1.69[a] | 0.087 | < 0.01 |
| Isobutyrate | 0.82 | 0.77 | 0.82 | 0.80 | 0.071 | 0.83 |
| Isovalerate | 1.21 | 1.39 | 1.43 | 1.29 | 0.130 | 0.17 |
| (A+B):P | 2.80 | 3.05 | 2.86 | 2.79 | 0.210 | 0.08 |

[a-b]Means within a row that do not have a common superscript differ at $P < 0.05$.
[1]Control = TMR without protein supplement; SRUT = TMR with slow released urea (Optigen, Alltech Inc., Nicholasville, KY); YMPT = TMR with yeast-derived microbial protein (DEMP, Alltech Inc.); and SYT = TMR with slow released urea and yeast-derived microbial protein.
[2]Expressed as mol/100 mol.

## Utilization of N and MCP Production

Because of decreases in DMI with added protein supplements, N intake decreased in diets containing SRU and/or YMP (Table 5). However, feeding YMPT tended ($P = 0.09$) to have the greatest milk N output, leading to an increase in efficiency of feed N-to-milk

N ratio compared with the control (0.31 vs. 0.25, respectively). While decreased N intake was the main reason for the increased efficiency, the fact that YMP is a RUP source and is able to escape ruminal fermentation and provide a high-quality AA may have also affected N efficiency for milk production. However, inconsistent results have been reported in many studies investigating supplementation of varying RUP sources. For instance, supplying different amounts and sources of RUP have been attributed to the depression of MCP synthesis or the inability of the RUP to provide the limiting AA (Cunningham et al., 1996).

Milk N-to-N intake ratio increased when cows were supplemented with SRU or YMP (Table 5), resulting in a negative correlation ($R^2 = 0.45$) between N intake and milk N efficiency (Figure 6). These results indicate that cows with less N intake are more efficient in utilizing the N and partitioning it toward milk TP production. There is a body of evidence to indicate that milk N efficiency is decreased, when N intake is increased (Castillo et al., 2000; Kalscheur et al., 2006; Dijkstra et al., 2013). Dijkstra et al. (2013) reported a negative relationship between N concentration in the diet and milk N efficiency. Castillo et al. (2000) reported that there was a positive relationship between N intake and milk N up to 400 g N intake/d, but above 400 g N intake/d, there was a negative relationship. In addition, Kalscheur et al. (2006) tested diets with increasing concentrations of RDP but a constant concentration of RUP, and found that milk N efficiency increased, as CP intake decreased ($R^2 = 0.28$). The increase in milk N efficiency when CP intake decreased was significant only in the lowest level of RDP concentration (6.80% DM), but not when cows were fed with higher concentrations of

RDP (8.20, 9.60, 11.0% DM; Kalscheur et al., 2006). The authors attributed the increased milk N efficiency in the low RDP diet to the high efficiency of the RUP (Kalscheur et al., 2006). Collectively, all the previous reports highlight that feed N intake considerably affects N utilization efficiency as is in the current study. Jonker et al. (2002) reported that for every additional gram of N in the diet over the recommendation decreases N utilization efficiency by 0.05 percentage units. When dietary protein is in excess, N utilization efficiency decreases and leads to greater amounts of N losses into urine and feces (Tamminga, 1992).

Concentration of MUN increased when SRU was supplemented in the diet and was the greatest in SYT (Table 5). The increase in MUN resulted from a tendency ($P = 0.10$) for ruminal $NH_3$-N concentration to increase in diets supplemented with SRU compared with the control and YMPT. Inostroza et al. (2010) also reported an increase in MUN concentration from 12.4 to 13.2 mg/100 mL for the control and SRU-containing diets, respectively. Wattiaux et al. (2005) reported that MUN concentrations between 10 to 14 mg/100 mL are normal, and diets at approximately 16.5% CP were found to be associated with a MUN concentration of about 12 mg/100 mL which is considered an optimal situation to not limit milk production but avoid unnecessary urinary N losses. The SRU product tested in this study is designed to release ammonia slowly, but its degradation rate has shown to change depending on the type of diet. For example, Holder (2012) indicated that when SRU was fed to Holstein steers in high-forage diets, the rate and extent of ruminal degradation of SRU was increased compared with a high-concentrate diet. The author concluded that the increase in SRU degradation may have been due to

**Table 5.** Nitrogen utilization of lactating Holstein dairy cows fed alfalfa hay-based TMR without or with protein supplements

| | Dietary treatments[1] | | | | | |
|---|---|---|---|---|---|---|
| Item | Control | SRUT | YMPT | SYT | SEM | *P* |
| N intake, g/d | 785[a] | 699[b] | 675[b] | 718[b] | 26.4 | 0.01 |
| Milk N, g/d | 193 | 195 | 204 | 193 | 8.3 | 0.09 |
| Milk N:N intake[2] | 0.25[b] | 0.28[a] | 0.31[a] | 0.27[ab] | 0.016 | 0.03 |
| MUN, mg/100 mL | 12.2[b] | 13.3[ab] | 12.4[b] | 13.7[a] | 0.52 | 0.01 |
| $NH_3$-N[3], mg/100 mL | 7.88 | 9.88 | 7.52 | 9.06 | 1.528 | 0.10 |
| Urinary N excretion,[4] g/d | 233 | 253 | 236 | 260 | 11.7 | 0.08 |
| Fecal N excretion,[5] g/d | 358[a] | 269[b] | 235[b] | 264[b] | 26.9 | < 0.01 |
| Manure N excretion,[6] g/d | 592[a] | 522[b] | 471[b] | 524[b] | 27.9 | 0.01 |
| UN:FN[7] | 0.65[b] | 0.94[ab] | 1.00[a] | 0.98[a] | 0.117 | 0.04 |
| MkN:MaN[8] | 0.34[b] | 0.38[ab] | 0.46[a] | 0.38[b] | 0.033 | 0.04 |
| MCP,[9] g/d | 2180 | 2126 | 2146 | 2177 | 124.5 | 0.94 |

[a-b]Means within a row that do not have a common superscript differ at $P < 0.05$.
[1]Control = TMR without protein supplement; SRUT = TMR with slow released urea (Optigen, Alltech Inc., Nicholasville, KY); YMPT = TMR with yeast-derived microbial protein (DEMP, Alltech Inc.); and SYT = TMR with slow released urea and yeast-derived microbial protein.
[2]Efficiency of use of feed N to milk N.
[3]Ruminal ammonia-N.
[4]Predicted using the equation: 0.026 × MUN, mg/100 mL × BW, kg (Wattiaux and Karg, 2004).
[5]Predicted using the equation: N intake, g/d – urinary N excretion, g/d – milk N, g/d.
[6]Manure N, g/d = urinary N excretion, g/d + fecal N excretion, g/d.
[7]UN:FN = urinary N to fecal N ratio, where urinary N and fecal N are expressed in g/d.
[8]MkN:MaN = milk N to manure N ratio, where milk N and manure N are expressed in g/d.
[9]Microbial protein production, g/d = ({[purine derivatives production − (0.385 × $BW^{0.75}$)]/0.85} × 70 × 6.25)/(0.13 × 0.83 × 1,000) (Janicek et al., 2008).

the increase in microbes that produce urease. The optimal ruminal pH for urease activity was reported to be between 6.8 and 8.5 (Muck, 1982). Although $NH_3$-N concentration tended to increase ($P < 0.10$) with SRUT compared with control and YMPT (9.88 vs. 7.70 mg/100 mL, respectively), supplementing SRU would not interfere with N metabolism, as the $NH_3$-N concentration was within its typical range at 1.3-28.9 mg/100 mL reported in lactating dairy cows (Kang-Meznarich and Broderick, 1981). The lower

ruminal $NH_3$-N concentration in YMPT compared with SRU-supplemented treatments may be attributed to the fact that YMP acts as a RUP escaping microbial degradation in the rumen.

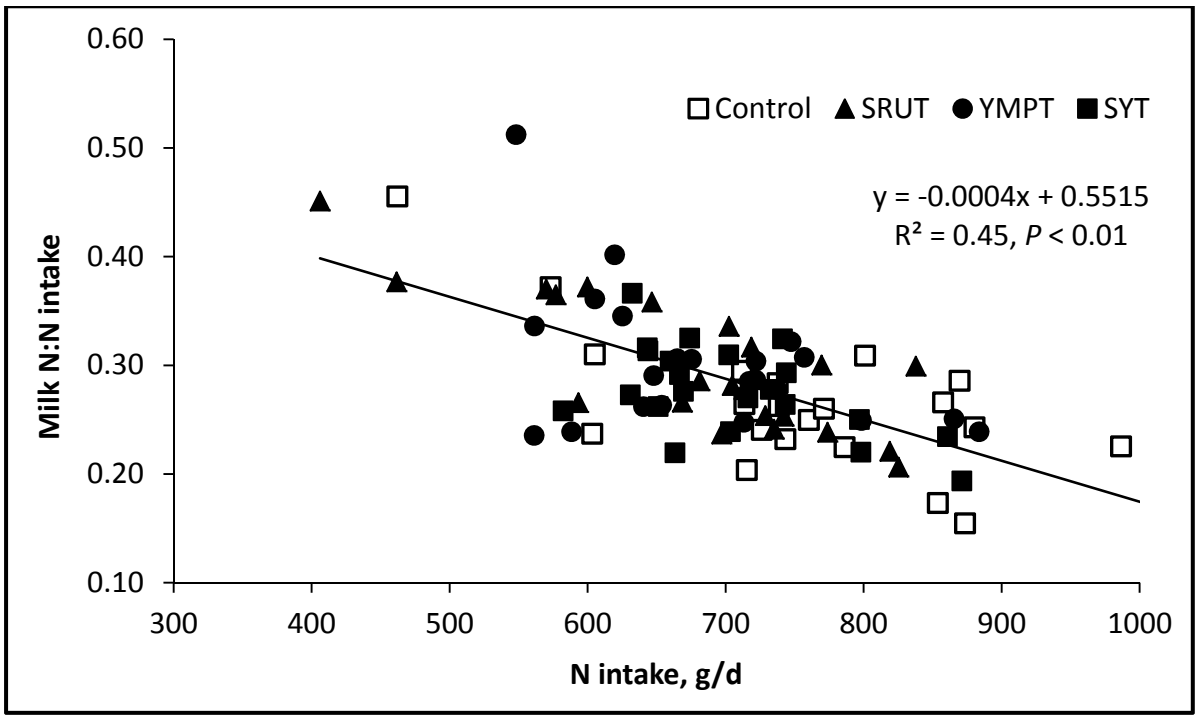


**Figure 6.** The relationship between N intake and efficiency of use of feed N to milk N (milk N:N intake) of lactating Holstein dairy cows fed alfalfa hay-based TMR without or with protein supplements. Control = TMR without protein supplement; SRUT = TMR with slow released urea (Optigen, Alltech Inc., Nicholasville, KY); YMPT = TMR with yeast-derived microbial protein (DEMP, Alltech Inc.); and SYT = TMR with slow released urea and yeast-derived microbial protein. Each point represents a value from 2 days from each period for an individual cow (n = 88).

Urinary N excretion tended ($P = 0.08$) to increase in SRUT and SYT (Table 5). Feeding protein-supplemented diets decreased fecal and manure N excretions compared with the control. Urinary N-to-fecal N ratio increased with protein supplementation mainly due to a sizable decrease in fecal N excretion with slight impact on urinary N excretion. Urinary N is known to be the most environmentally volatile N (Varel et al.,

1999), because in the environment microbial ureases react with urinary N (Muck, 1982), and then urea is rapidly hydrolyzed to ammonia and volatilized into the environment (James et al., 1999). The tendency ($P = 0.08$) for decreased urinary N excretion in YMPT demonstrates an additional benefit of YMP supplementation on the environment over SRU supplementation.

Increased milk N-to-manure N ratio was observed in YMPT (0.46) compared with the control and SYT (0.34 and 0.38, respectively; Table 5). Cows fed the control excreted overall more manure N because of the increase in DMI compared with the other treatments. As N intake increases in lactating dairy cows, manure N output also increases (Yan et al., 2006). A higher milk N-to-manure N ratio indicates that less manure N must be managed per unit of milk N produced by the herd, leading to a desirable N management practice on-farm (Holt et al., 2013). The protein in AH is extensively broken down in the rumen and used inefficiently (Broderick et al., 1992), increasing the risk for excess N to be released into the environment. However, supplementation of YMP in high-forage diets with a high concentration of AH can increase the utilization efficiency of the N to be converted to milk N rather than manure N, resulting in positive impacts on the environment.

Dietary treatments did not influence MCP yield (Table 5). Yield of MCP is determined by 2 factors: the efficiency of utilization of energy from fermented OM by ruminal microbes and total OM fermented in the rumen. Energy-yielding substrates supplied from starches, sugars, fiber, and organic acids are considered to be the most important for MCP yield (Clark et al., 1992). Although cows fed protein-supplemented

diets decreased DMI, decreased passage rate of digesta in the rumen may have increased ruminal fermentability of energy-yielding substrate, causing no difference in MCP yield across dietary treatments. Feed intake affects ruminal digestion and passage rate (Yang et al., 2002). When DMI is decreased, passage rate is also decreased, leading to an increase in digestion and influencing MCP synthesis (Shaver et al., 1988).

Ruminal $NH_3$-N concentration is a result of balance between production (proteolysis) and assimilation (De Visser et al., 1997), and thus any efforts to maximize N utilization in the rumen should involve an optimal balance between the 2 metabolic processes. There has been much debate about optimal concentration of ruminal $NH_3$-N concentration due to its impacts on MCP synthesis. Satter and Slyter (1974) suggested 5 mg $NH_3$-N/100 mL for a safety margin for MCP yield. In contrast, Mehrez et al. (1977) observed that in situ barley DM digestion increased with increasing $NH_3$-N concentration until reaching at 20 mg/100 mL when urea was infused into the sheep rumen, whereas Odle and Schaefer (1987) observed maximal rates of in situ DM disappearance at 12 and 6 mg $NH_3$-N/100 mL in cattle for barley and corn, respectively. Although feeding SRUT decreased intakes of DM and nutrients compared with control in the current study, the increased $NH_3$-N concentration with feeding SRUT may have stimulated MCP yield under the reduced nutrient intake, resulting in a similar MCP yield compared with control (2126 vs. 2180 g/d; $P > 0.10$). Cellulolytic bacteria prefer or require N in the form of ammonia (Russell et al., 1992), and thus, the likely contribution of SRU to MCP yield may have resulted in increased feed efficiency compared with control. Meanwhile, it may be beneficial to supplement YMP in high-forage diets or when cows have low ME intake, because YMP

does not require energy to be converted in the rumen to MCP, but rather escapes the rumen in the liquid fraction while still providing the essential AA needed for milk production. In a study performed by Maxin et al. (2013) where they compared different protein sources (SBM, canola meal, high-protein dried distiller's grains, or wheat dried distiller's grains with solubles), the authors found no differences between treatments in MCP yield (averaging 2240 g/d). In the study, the authors stated that changes in supply of AA should first be explained by the difference in RUP fraction instead of the effects of MCP synthesis (Maxin et al., 2013). Milk protein secretion in dairy cows is closely associated with the supply of MP (NRC, 2001), which consists of the intestinally absorbable dietary protein and intestinally absorbable MCP. Because MCP yield was similar across dietary treatments, it is clear that increased TP yield due to feeding YMPT (Table 3) would be a direct result of increased AA supply from YMP to the small intestine.

**Economic Perspective**

While feeding SRUT resulted in the lowest feed cost at $0.340/kg DM, feeding YMPT had the greatest feed cost compared with all other treatments (Table 6). When IOFC was calculated based on milk yield, there was a tendency ($P = 0.10$) for SRUT to have the greatest increase in IOFC ($1.11) compared with the control. The IOFC based on milk component yields resulted in a tendency ($P = 0.09$) for YMPT to have the greatest increase of $1.32 compared with the control. The difference in IOFC between the treatments is mostly due to the decrease in DMI in the treatments supplemented with

SRU and/or YMP, and the tendencies ($P < 0.10$) for increased yields of milk and milk components (milk fat and TP; Table 3) compared with the control. Feed costs can account for more than 50% of total costs on a dairy (Phuong et al., 2013). Reducing feed requirements and feed costs can be a major contributor for improving dairy profitability (Connor et al., 2012), because although it generally costs more to feed high producing cows, the increased productivity and efficiency lead to improved profitability (VandeHaar, 1998). Although supplementing YMP increased feed costs, feeding YMPT increased nutrient utilization and feed efficiency, resulting in increased production and IOFC, making YMP a profitable protein supplement for high-forage lactation rations with a greater concentration of AH.

**Table 6.** Income-over feed cost (IOFC) of lactating Holstein dairy cows fed alfalfa hay-based TMR without or with protein supplements

| | Dietary treatments[1] | | | | | |
|---|---|---|---|---|---|---|
| Item | Control | SRUT | YMPT | SYT | SEM | *P* |
| Feed cost, $/kg DM | 0.345 | 0.340 | 0.363 | 0.359 | - | - |
| IOFC, $/d/cow | | | | | | |
| Milk yield[2] | 6.61 | 7.72 | 7.64 | 7.01 | 1.016 | 0.10 |
| ECM yield[3] | 6.82 | 8.22 | 8.36 | 7.41 | 0.936 | 0.11 |
| Milk component yield[4] | 6.19 | 7.43 | 7.51 | 6.59 | 0.812 | 0.09 |

[1]Control = TMR without protein supplement; SRUT = TMR with slow released urea (Optigen, Alltech Inc., Nicholasville, KY); YMPT = TMR with yeast-derived microbial protein (DEMP, Alltech Inc.); and SYT = TMR with slow released urea and yeast-derived microbial protein.
[2]IOFC calculated on total milk yield (kg/d).
[3]IOFC calculated on ECM yield (kg/d).
[4]IOFC calculated on the value of milk components (fat, true protein, and lactose, kg/d).

# CONCLUSIONS

Greater emphasis has been placed on devising a practical approach of enhancing milk protein production as well as reducing dietary N wastes in the dairy industry for many years. Although DMI was decreased when SRU and/or YMP were supplemented in high-forage diets, the likely increased supply of AA in YMP during low energy intake may allow for more energy to be partitioned toward milk production instead of BW gain, improving milk protein yield, feed efficiency, and overall lactational performance. Also, increased milk N-to-manure N ratio by feeding YMPT provides benefits for the environment through better feeding and management programs, while at the same time achieving acceptable N utilization efficiency and N excretion. However, we have yet to explore the interactions of AA and energy-substrate metabolism in the mammary gland and whole body, which may contribute to identifying additional evidence for the positive effects of supplementing YMP in high-forage lactation diets. This study suggests that replacing SBMCM in high-forage rations containing a high proportion of AH with YMP during early lactation enhanced nutrient utilization and efficiency, leading to increased milk and milk protein production and decreased potential negative environmental impacts and improved dairy profitability due to greater IOFC.

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