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Lactational Performance of Dairy Cows Fed Yeast Derived Microbial Protein in Low and High Forage Diets

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LACTATIONAL PERFORMANCE OF DAIRY COWS FED YEAST DERIVED MICROBIAL PROTEIN IN LOW AND HIGH FORAGE DIETS

BY

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This thesis is approved as a credible and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AA= amino acid

ADIN= acid detergent insoluble nitrogen

Ala= alanine

Arg= arginine

Asn= asparagine

Asp= aspartate

BCS= body condition score

BW= body weight

CCP= cell-penetrating peptides

CP= crude protein

DM= dry matter

DIM= days in milk

DMI= dry matter intake

EAA= essential amino acid

ECM= energy corrected milk

FCM= 3.5% fat corrected milk

FSNAN= feed soluble non ammonia nitrogen

Gln= glutamine

Glu= glutamate

Gly= glycine

His= histidine

Ile= isoleucine

Leu= leucine

Lys= lysine

Met= methionine

MCP= microbial crude protein

MP= metabolizable protein

MUN= milk urea nitrogen

N= nitrogen

NAN= non ammonia nitrogen

NDF= neutral detergent fiber

NDIN= neutral detergent insoluble nitrogen

NEAA= nonessential amino acid

NPN= non protein nitrogen

NRC= National Research Council

pef= physically effective fiber

Phe= phenylalanine

Pro= proline

PSPS= Penn State Particle Separator

RDP= rumen degradable protein

RUP= rumen undegradable protein

Ser= serine

SNF= solids not fat

Tau= taurine

Thr= threonine

TMR= total mixed ration

TS= total solids

Trp= tryptophan

Tyr= tyrosine

Val= valine

VFA= volatile fatty acids

YMP= yeast-derived microbial protein

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

EFFECT OF YEAST DERIVED MICROBIAL PROTEIN IN LOW AND HIGH FORAGE DIETS ON THE PERFORMANCE OF DAIRY COWS

Angela Kristia Manthey

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The objective of this study was to investigate the effect of substituting soybean meal products with yeast-derived microbial protein [(YMP) DEMP; Alltech Inc., Nicholasville, KY] in diets containing two forage concentrations. Sixteen Holstein cows (4 primiparous and 12 multiparous) were randomly assigned to a replicated 4 × 4 Latin square in a 2 × 2 factorial arrangement of treatments. Diets contained low (LF; 45% of diet DM) or high forage (HF; 65% of diet DM) and YMP at 0 (NYMP) or 2.25% (WYMP) of the diet. The forage mix consisted of 67% corn silage and 33% alfalfa hay (DM basis). There were no interactions of forage and YMP for any of the production parameters. Cows fed LF consumed more DMI (26.9 vs. 25.2 kg for LF and HF, respectively; P=0.004) and produced more milk (40.1 vs. 37.83 kg; P=0.005) than cows fed HF regardless of the addition of YMP. Milk fat percentage was lower in cows fed LF compared to HF (3.76 vs. 3.94; P=0.04), whereas fat yield tended to be lower (1.52 vs. 1.45 for NYMP and WYMP respectively; P=0.07 in cows fed NYMP . Although milk protein percentage did not differ between forage concentrations with or without the addition of YMP, protein yield and total solids were greater in cows fed LF. Cows fed LF produced more energy-corrected milk (ECM) than those fed HF (41.9 vs. 40.2; P<0.04). Feed efficiency (ECM/DMI) was greater for cows fed NYMP compared to WYMP (1.52

vs. 1.45; $P<0.02$). An interaction of forage and YMP was observed for propionate concentration, acetate and propionate proportion, and acetate:propionate ratio, as well as a tendency for an interaction of forage and YMP for ruminal pH. A forage effect was observed for ruminal ammonia, isobutyrate concentration, butyrate concentration, isovalerate concentration, as well as proportions of isobutyrate, butyrate, and isovalerate with cows on HF diets having greater concentrations and proportions of these measures. There were no differences in plasma glucose concentrations between forage or YMP levels. There was a forage effect on arterial and venous PUN concentrations. Cows fed the HF diets had greater PUN concentrations. There was a forage effect on the EAA including Ile, Leu, Lys, Met, Thr, and Val. Cows fed the LF diets had greater concentrations of these circulating arterial plasma AA than cows fed the HF diets ($P<0.05$). A forage effect on circulating concentrations of Ile and Val was observed with cows fed LF diets having greater concentrations of these AA than cows fed HF diets. Cows fed NYMP had greater circulating venous concentrations of Arg, His, Ile, Lys, and Val than cows fed WYMP. These cows also tended to have greater concentrations of circulating venous Thr than cows fed WYMP ($P=0.06$). The arteriovenous differences of Arg, Ile, Leu, Lys, Met, Phe, and Thr were greater in cows fed LF diets compared to cows fed HF diets. There was also a tendency for the interaction of forage and YMP for the extraction efficiency of Met. Results suggested that the forage level, as well as, YMP affected cow performance. Cows fed NYMP had greater milk fat yield as well as greater feed efficiency. Cows fed LF diets produced more milk and ECM, yet resulted in a lower fat percentage.

CHAPTER 1

LITERATURE REVIEW

Introduction

The cost of feed ingredients accounts for approximately 50 to 70% of the total cost of livestock production, and the price of feed ingredients keeps increasing with protein being one of the more expensive nutrients (Piao et al, 1998; Kondo et al., 2007). Therefore, dairy diets should be formulated to maintain high milk production with sufficient amounts of rumen degradable protein (RDP) and rumen undegradable protein (RUP) that meets the metabolizable protein (MP) requirements for production to ensure a high rate of nitrogen (N) use without feeding excess crude protein (CP) (Agle et al., 2010a). Microbial crude protein (MCP), the protein produced by rumen microbes, has a superior amino acid (AA) profile compared to the AA profile in commercial feed ingredients (Clark et al., 1992). Traditional feed ingredients cannot completely meet the AA requirements of high producing dairy cows. Feeding additional RDP may result in diminishing returns of MCP when N increases (Satter and Slyter, 1974). By maintaining adequate RDP in the diet, a high quality source of RUP can be included in the diet to impact the quantity of AA reaching the small intestine to be absorbed across the intestinal wall to sustain milk components and yield (Santos et al., 1998; Kalscheur et al., 2006).

Recent technology advances are being developed to find alternative protein supplements to replace traditional feeds, such as soybean meal (Seo et al., 2008). These newer technologies include; slow-release urea (Taylor-Edwards et al., 2009), rumen-protected AA (Ordway et al., 2009), oilseeds or oilseed meals that have been heat or chemically treated (NRC, 2001), and peptides (Gilbert et al., 2008). The escape protein

can increase MP and improve the AA profile of the MP reaching the small intestine (NRC, 2001).

New feed ingredients and technologies that will maximize production and economic return, while meeting environmental requirements are being developed. The recycling of industrial waste products into animal feed has been explored as a means to reduce environmental concerns (Seo et al., 2008). Feeding soluble protein and peptides could be possible alternatives. Fermentation and MCP production can be improved by the inclusion of peptides and free AA (Argyle and Baldwin, 1989; Chikunya et al.,1996). Low molecular weight peptides could also contribute to AA absorption from the small intestines after ruminal escape (Rémond et al., 2000).

Protein Utilization in Ruminants

Ruminants have very specialized digestive systems that set them apart from monogastrics. Their digestive systems are characterized by pregastric retention and fermentation, and as a result have a mode of digestion that allows better access to energy in fibrous feeds than other herbivores (Van Soest, 1994). Due to their unique digestive systems, ruminants can convert feedstuffs that monogastrics cannot use into valuable products, such as milk and meat, for human use. Ruminants can utilize poor quality protein, NPN, and N recycled in the rumen to produce a high quality MCP to support the demands of lactation.

It is the goal of nutritionists to provide the dairy cow with adequate RDP to maintain optimal rumen efficiency and obtain the desired productivity with a minimal amount of dietary CP (NRC, 2001). In order to do so, the N needs of the rumen microbes need to be met with RDP, but not exceeded in order for maximal MCP production to

occur. The production of milk protein can be increased by improving the profile of AA in MP (NRC, 2001). This can be done by decreasing excess dietary protein and increasing fermentable carbohydrates because MCP synthesis is driven by the availability of energy from carbohydrate fermentation. Therefore, nutrient synchrony, the synchronization of the rates of carbohydrate and RDP degradation in the rumen, is a strategy to optimize MCP synthesis (Cole et al, 2008). It is desirable to improve protein and N efficiency to reduce feed costs and the concern of N waste.

Historically, dairy diets had been balanced based upon digestible protein values (Thomas, 1971). Then, it was decided that protein requirements should be expressed as a percentage of CP because of the relationships between protein digestibility and protein or dry matter intake as well as other variables (Holter and Reid, 1959; Brown, 1966; Reid et al., 1966). In 1985, the concept of RDP and RUP was introduced (NRC, 1985). This separated CP into a ruminally degraded fraction, and a ruminally undegraded fraction (Ruminant Nitrogen Usage, 1985). At the time these fractions were referred to as DIP and UIP respectively. The concept of absorbable protein was introduced in 1989 (NRC, 1989). Later, in 2001, the concept of MP was introduced (NRC, 2001). Now, RDP and RUP are taken into account when formulating diets for protein. First, the diet must provide sufficient RDP to meet the requirements of the ruminal microorganisms to maximize microbial protein synthesis. The MP protein requirement is met with supplemental RUP when microbial protein synthesis alone is insufficient to meet the MP requirements.

Protein Requirements for Lactation

The amount of protein required for lactation depends upon the amount of protein that is secreted in the milk (NRC, 2001). Milk production can easily be manipulated by dietary CP concentrations as demonstrated by research conducted throughout the years (Kwan et al, 1977; Cressman et al., 1980; Kung and Huber, 1983; Colmenero and Broderick, 2006). In rations containing less than 12% CP, milk production decreased compared with those fed diets with greater CP concentrations (Kwan et al., 1977). Studies on milk response were conflicting when diets contained 12 to 14% CP. Cressman et al. (1980) fed diets ranging from 12.2 to 17.7% CP and found that milk production, 32.7 vs. 29 kg, was greater for multiparous cows fed 12.2% CP compared to cows fed 15.1% CP respectively. The opposite was discovered by Colmenero and Broderick (2006) who investigated the effects of feeding increasing levels of dietary CP ranging from 13.5 to 19.4%. As dietary CP increased, 3.5% fat corrected milk (FCM) tended to linearly increase (P=0.10). Kung and Huber (1983) also found that milk production increased with increased dietary CP concentration when lactating dairy cows were fed diets containing 11.3, 14.5, and 17.5% CP. Edwards et al. (1980) used similar treatments and compared rations containing 13, 15, and 17% CP. The results were similar to those of Kung and Huber (1983). Cows fed the 15 and 17% CP rations produced approximately 3.2 kg/d more milk than those fed 13% CP, but there were no differences in production between the 15 and 17% CP diets.

Research has demonstrated that CP concentration affects DMI (Grieve et al., 1974; Murdock and Hodgson, 1978; Cressman et al., 1980; Kung and Huber, 1983). When diets contained greater than 14% CP, milk production increased; however, DMI also increased, which resulted in decreased efficiency of protein use (Grieve et al., 1974;

Murdock and Hodgson, 1978). Kung and Huber (1983) also reported that DMI increased with increased dietary CP level when lactating dairy cows were fed diets containing 11.3, 14.5, and 17.5% CP. Greater differences in milk production and DMI were observed between the 11 and 14% CP than the 14 and 17% CP. Cressman et al. (1980) reported a quadratic effect on DMI for multiparous for cows fed rations containing 12.2, 15.1, and 17.7% CP. The cows consumed 18.0, 15.7, and 19.9 kg/d of DM, respectively.

Multiparous and primiparous cows respond differently to dietary CP levels. Increases in milk production and solids not fat (SNF) of multiparous cows were observed when dietary CP was increased from 12.2 to 17.7% by substituting soybean meal for high moisture ear corn (Cressman et al., 1980). Although there was an increase in milk production for multiparous cows when dietary CP was increased, there were no differences in milk production for primiparous cows. A similar response was shown by Roffler et al. (1978) where multiparous, but not primiparous cows showed an increase in milk production when dietary CP increased from 12.2 to 16.2%. The response to protein supplementation in milk production between primiparous and multiparous cows was not explained, but could possibly be explained by the fact that the primiparous cows are still growing.

Diminishing returns in milk production to increases in dietary CP is the response most documented by many meta-analyses (Roffler et al., 1986, NRC, 2001, Ipharraguerre and Clark, 2005). The NRC (2001) conducted a multivariate regression analysis of 82 protein studies. This analysis yielded an equation to predict milk production responses of 0.75 kg/d when CP was increased from 15 to 16% and 0.35 kg/d when CP increased from 19 to 20% (NRC, 2001). Maximum milk production in this study was found when diets

contained 23% CP. When Ipharraguerre and Clark (2005) conducted a similar summary of 112 studies using different methodology, estimations predicted an increase of 0.94 and 0.42 kg/d when dietary CP was increased from 15 to 16 and 19 to 20% respectively. Maximum milk production in this study was achieved with diets containing 22.8% CP (Ipharraguerre and Clark, 2005).

Roffler and others (1986) studied the response of increased concentrations of dietary CP on milk yield and DMI of early lactation cows. This data set included 17 published studies having a concentration range in CP was from 9.5 to 20.2% achieved by varying the amount of soybean meal in the diet. The model predicted that milk yield changes as a result of increased CP concentration. Increasing the CP concentration from 12 to 13% would result in 1.6 kg/d of milk yield and 0.7 kg/d of DMI, and increasing the CP from 18 to 19% would increase milk yield 0.2 kg/d and DMI less than 0.1 kg/d (Roffler et al., 1986). In addition to diminishing returns in milk production, this study also demonstrated diminished responses in DMI to increased dietary CP.

Although milk production may be increased by feeding increased CP concentrations, unfavorable effects on efficiency of nutrient utilization, the environment, and the overall profit of the dairy operation may occur (NRC, 2001). Colmenero and Broderick (2006) observed linear and quadratic trends for milk yield and FCM, respectively as dietary CP increased from 13.5 to 15, 16.5, 17.9, and 19.4% of DM. Protein and fat yields also showed linear and quadratic trends (P=0.06 and P=0.09 respectively), both parameters peaking at 16.5% CP and no further improvements were observed at greater dietary CP concentrations (Colmenero and Broderick, 2006). However, concentrations of blood urea N and MUN increased in response to CP

concentration. As CP concentration increased, there was a linear decline in apparent N efficiency (milk protein N/N intake), with the 16.5% CP protein level having the greatest N efficiency.

Earlier nutritional models have balanced diets by CP concentration in order to meet the N requirements of the cow (Reid et al., 1966; Thomas, 1971). However, the CP balance does not account for the amount of NPN, the rate and extent of ruminal degradation, and the intestinal digestibility and AA composition of RUP (NRC, 2001). Therefore, it is very important to further break down the protein requirements of the dairy cow down based upon RDP and RUP.

Effect of Rumen Degradable Protein on Microbial Crude Protein Synthesis

Ruminally degradable protein, as the name suggests, is acted upon by ruminal microbes and is degraded in the rumen where it becomes a substrate for microbial growth and the synthesis of MCP. It is comprised of non-protein nitrogen (NPN) and true protein N (Bach et al., 2005). The NPN is regarded as the N in ammonia, AA, and small peptides that are readily and completely degraded in the rumen through microbial metabolism (NRC, 2001; Bach et al., 2005). The true protein is further degraded to peptides and AA and is eventually converted to ammonia or MCP.

In order to first degrade protein within the rumen, bacteria must first attach to feed particles, followed by activity of cell-bound microbial proteases (Brock et al., 1982). The peptides and AA are then transported inside microbial cells where it may be directly transaminated into MCP in the presence of adequate energy such as carbohydrates (Bach et al., 2005). If energy is not available, the AA can then be deaminated and fermented to VFA, CO_2, and ammonia (Tamminga, 1979).

Several factors affect ruminal protein degradation. The most important being the type of protein degraded, synergistic relationships with other nutrients such as carbohydrates, and the prevailing microbial population within the rumen. The solubility of the protein determines its susceptibility to microbial proteases and therefore its ruminal degradability (Bach et al., 2005). Prolamins and glutelins are slowly degraded; however, globulins are soluble and readily degradable (Romagnolo et al., 1994). Protein degradation is inversely related to the passage rate through the rumen (Orskov and McDonald, 1979). These changes are small and only represent a small increase of RUP supply to the small intestine (Bach et al., 2005). Within the rumen, the optimal pH ranges from 5.5 to 7.0; however, in an environment on the lower end of the pH scale, protein degradation decreases (Kopecny and Wallace, 1982).

The MCP that is synthesized within the rumen supplies most of the AA that pass to the small intestine (NRC, 2001). Of the non-ammonia nitrogen (NAN) that enters the small intestine, MCP comprises approximately 40% in high protein diets, 60% in low protein diets, and 100% in purified NPN diets (Church, 1988). Clark et al. (1992) similarly found in a summary of 152 different dietary treatments that microbial N supplied an average of 59% of the NAN that passed to the small intestine, with a range of 34 to 89%. The greater percentages were attributed to the cows being fed all forage diets containing greater concentrations of NPN in the CP.

In order to optimize microbial growth, diets must be properly balanced to provide adequate N and energy. The synthesis of MCP is limited by the energy available for microbial fermentation and the efficiency in which that energy is used (NRC, 2001). As long as carbohydrates are not limiting, theoretically, bacterial N and bacterial efficiency

should continue to increase as RDP increases in the diet (Stokes et al., 1991). Kalscheur et al. (2006), however, found that formulating diets to meet, but not exceed the RDP requirement of the microbes optimized microbial growth, reduced N excretion, and improved overall N use by the cow.

Effect of Rumen Undegradable Protein in Dairy Cow Diets

The MCP and feed protein that bypasses or escapes ruminal degradation reaching the small intestine for absorption is RUP (NRC, 2001). Additional protein of great quality may need to be supplied to the small intestine, as MCP itself may not be sufficient to meet the protein requirement if production is high. Substituting RDP with RUP is not recommended because it can decrease MCP synthesis, if RDP becomes limiting (Santos et al., 1998).

Various technologies have been explored to provide an increased flow of unaltered protein to the abomasum, while having optimal digestibility in the intestine (NRC, 2001). Many of the methods to decrease the ruminal degradation of protein that have been developed include heat, chemical agents, or a combination of both (Satter, 1986; Broderick et al., 1991). The challenge has been to justify the cost of the treatment, optimizing ruminal bypass while maintaining minimal intestinal absorption AA loss. Heat processing denatures proteins by the formation of protein-carbohydrate (Maillard reactions) and protein-protein crosslinks (NRC, 2001). While heat treating, careful quality control must be implemented to ensure that overheating or under-heating the feeds does not occur. The heating conditions must be controlled to optimize the content of digestible RUP without forming indigestible Maillard products that may only deliver

small concentrations of RUP, which is observed in overheating and under-heating situations, respectively (Schwab, 1995).

The chemical treatment of feeds is divided into three categories: chemicals that combine with and introduce cross-links within the proteins, chemicals that alter protein structure by denaturation, and chemicals that bind to proteins with little or no alteration of protein structure (Broderick et al., 1991). Chemical treatments have not been well received commercially and are usually combined with some form of heating to alter the protein structure.

A comprehensive review was conducted by Santos et al. (1998) to investigate the effects of RUP on cow performance. In 22 of 29 comparisons from 15 different metabolism studies, diets high in RUP decreased MCP synthesis. In the same review Santos et al. (1998) also looked at 127 comparisons in 88 lactation studies where soybean meal was substituted by high sources of RUP, milk yield was slightly greater when the source of RUP was fish meal or treated soybean meal (Santos et al., 1998). When considering limiting AA such as Lys and Met, the quality of the RUP source also played a role. Under these circumstances, fish meal is considered a high quality RUP source, while corn gluten meal a low quality source. Fish meal contains a good balance of Lys and Met, whereas corn gluten meal contains a good supply of Met but is low in Lys (Santos et al., 1998). In nine comparisons when fish meal was compared to corn gluten meal, fish meal increased milk production, decreased milk fat percentage, and did not change milk protein percentage (Santos et al., 1998).

Past studies have demonstrated that there is variability in the response of RUP supplements (Ipharraguerre and Clark, 2005). Ipharraguerre and Clark (2005) conducted

a review that attributed the variability of response to the quantity and quality of protein of the control diet and the RUP source of the experimental diets. The CP percentage of the diets also played a lesser role in the variability of cow performance, as well as, the amount and source of RUP in the experimental diets. When comparing soybean meal and fish meal, fish meal yielded the greatest improvement in milk protein yield, but depressed milk fat yield (Ipharraguerre and Clark, 2005). The greatest increase in milk production was provided by treated soybean products, which increased production by approximately 3% (Ipharraguerre and Clark, 2005).

Previous work evaluated that RUP content of the true protein source affects cow performance. Brito and Broderick (2007) conducted a study in which urea was substituted with sources of true protein such as solvent soybean meal, cottonseed meal, and canola meal) to yield isonitrogenous diets (16.5% CP) with different concentrations of RDP and RUP. If the dietary RUP content is increased too much, it could lead to an inadequate RDP supply that will affect the profile of the absorbed AA and as a result milk production as well. Therefore, the objectives of the study were to compare the effects of supplementing CP as urea or 1 of 3 true protein sources differing in RUP content and AA profile on the production, N utilization, nutrient digestibility, and ruminal metabolism of lactating dairy cows. The concentrations of RDP for the urea, solvent soybean meal, cottonseed meal, and canola meal were 13.1, 11.0, 10.7, and 11.5% of DM, respectively, whereas RUP concentrations were 3.16, 5.53, 6.03, and 5.15% of DM, respectively as estimated by the NRC (2001). The cows fed the urea diet had lower milk yield, milk components, and feed and N efficiency (milk N/N intake) compared to the solvent soybean meal, cottonseed meal, and canola meal diets. This diet

also had the greatest RDP and lowest RUP concentrations. The yields of fat and protein were lower on cottonseed meal than on canola meal, and were intermediate on solvent soybean meal. The urinary N and N efficiency were also reduced on the cottonseed meal diet suggesting that there was poorer intestinal digestion or the AA pattern of absorbed protein negatively affected utilization of the cottonseed meal (Brito and Broderick, 2007). Therefore, the RDP and RUP concentrations are important, as well as, the composition and digestibility of each fraction.

Ruminal Protein Degradation Kinetics

The degradation of protein in the rumen is described by first-order mass action models (NRC, 2001). These models assume that the CP of the feedstuffs contains multiple fractions that vary in rate of degradation and ruminal disappearance (NRC, 2001). The model that divides in situ ruminal protein degradation into fractions A, B, and C is the most used (Orskov and McDonald, 1979). The rate of ruminal degradation of fraction A, the soluble N fraction, is assumed to be infinite (Reynal et al., 2007). The portion of dietary protein that is potentially degraded in the rumen (fraction B) is assumed to be used in microbial protein synthesis or production of ammonia and carbon skeletons (Reynal et al., 2007). If a portion of these fractions (A or B) escapes ruminal degradation as soluble protein, peptides, and free AA, these assumptions may not be true (Reynal et al., 2007). Fraction C is the protein that is ruminally undegradable.

In dairy cows fed alfalfa or silage based diets, the feed-soluble non-ammonia N (FSNAN) comprised 30 to 35% of the total N pool in the rumen and had a very high outflow rate (Hristov et al., 2001). It has also been reported that a significant component of the flow of NAN entering the omasal canal was due to the contribution of FSNAN

(Choi et al., 2002; Reynal et al., 2007). This suggests that not all of the FSNAN is degraded and utilized in the rumen, as the models assume from degradation kinetics. Additionally, the passage rate of feedstuffs with high proportions of FSNAN should be considered equal to that of the liquid phase of the rumen.

Metabolizable Protein in Dairy Cow Diets

Metabolizable protein (MP) is the net quantity of true protein or AA absorbed in digestion. In ruminants this consists of the true protein that is digested postruminally and the resulting AA that are absorbed by the intestine (NRC, 2001). The cow's MP supply can be defined as the sum of the MCP that flows to the small intestine and the dietary protein that escapes from the rumen undegraded and is absorbed in the small intestine. Endogenous sources also contribute to MP. Twenty percent of MCP is considered to be provided by nucleic acids, while the other 80 percent is considered to be true protein provided by bacteria and protozoa (NRC, 2001). The true protein of MCP is considered to be 80 percent digestible, therefore the conversion of MCP to MP is assumed to be 64 percent (NRC, 2001). The ruminally undegradable feed CP is assumed to be 100 percent true protein and estimates of intestinal digestibility of the RUP fraction of feedstuffs vary from 50 to 100 percent (NRC, 2001). Therefore, the contribution of RUP to MP is variable and feed type must be considered. There is very limited data that has been published regarding the digestibility of true protein in endogenous CP, the true protein of endogenous CP passing to the small intestine is assumed to be 50 percent (NRC, 2001). The true protein of endogenous CP is assumed to be 80 percent digestible and as a result, the conversion of endogenous CP to MP is thought to be 40 percent (NRC, 2001).

Metabolizable protein concentration affects cow performance. Wright et al. (1998) reported a linear increase in milk and protein production at the expense of N efficiency when MP concentration in the diet increased. The concentration of MP in the diet was increased by increasing RUP while keeping the RDP consistent. A study was conducted by Wang et al. (2007) to investigate the effects of MP on milk production of mid-lactation cows to evaluate the supplementation of MP as a way to increase the AA entering the small intestine. As MP increased in the diets by increasing the RUP concentration, there was a linear decrease in N efficiency because the N was not utilized as efficiently and was excreted in the urine. There was also a linear increase in milk yield, and quadratic effects on milk protein, fat, total solids, and SNF. In another study, the importance of MP balance on milk production of mid- lactation cows was studied with diets that decreased in CP concentration (Agle et al., 2010a). Three diets with decreasing CP (15.4, 13.4, and 12.9%) and RDP concentrations (10.3, 8.4, and 7.1%), delivered RDP balances of 162, -326, and -636 g/d and MP balances of 323, -44, and 40 g/d respectively. There were no differences in milk yield (30.9 kg/d; P=0.45) and components between treatments, but MUN and N excretion in the manure and urine were less for the low and medium CP diets than the high CP diet. This showed that diets with reduced CP and RDP concentrations yielded manure with lower ammonia-emitting potential and did not negatively affect cow performance when the cow's MP requirements were met (Agle et al., 2010a).

Effect of Dietary Protein on Reproduction Performance

Overfeeding protein can have a negative effect on reproduction (Blanchard et al., 1990; Butler et al., 1996). This is especially applicable when RDP is overfed (Ferguson

and Chalupa, 1989). The excess RDP can establish an energy toll on the animal because the animal must work to degrade the excess protein (Tyrrell et al. 1970). The excess RDP gets broken down into ammonia by rumen microbes in the rumen (McCormick et al., 1999). The ammonia then passes through the rumen epithelium and is converted to urea in the liver (McCormick et al., 1999). This results in lower conception rates due to increased plasma urea nitrogen levels (Canfield et al., 1990, Butler et al., 1996, and McCormick et al., 1999). There are three theories that are used to explain how dietary protein suppresses fertility: 1) the dietary protein directly affects the uterine environment, 2) alterations in gonadotropin or progesterone secretion, and 3) imbalances in protein:energy relationships (Canfield et al., 1990). Some previous researchers; however, have not observed a negative relationship between protein and reproduction (Howard et al., 1987; Guo et al., 2004).

Added RUP promotes reproductive efficiency if it replaces RDP, by decreasing the amount of plasma urea nitrogen, which can improve reproduction (Butler et al, 1996). It is able to do this because the RUP escapes degradation by microbes in the rumen which may reduce ammonia N availability, making the RUP available as AA or peptides in the small intestine. Besides just influencing reproductive efficiency, increased RUP also promotes increased milk production because it provides the small intestines with added AA needed to enhance milk production (McCormick et al., 1999).

Maintaining low concentrations of CP in the diet has been shown to increase plasma progesterone concentrations in dairy cows (Sonderman et al., 1987). Strong concentrations of plasma progesterone, such as 5 to 9 ng/mL, during the luteal phase of at least one estrous cycle before insemination were hypothesized to increase the incidence

of conception (Folman et al., 1973). Therefore by keeping dairy cattle on a plane of nutrition that does not overuse protein, conception may increase as a result of plasma progesterone. Progesterone levels are also important in maintaining pregnancy. The elevated plasma progesterone levels are beneficial in improving embryo survival (Law et al. 2009).

Amino Acids in Dairy Cattle Nutrition

Absorbed AA are the building blocks for the synthesis of tissue and milk proteins (NRC, 2001). Amino acids can be precursors for gluconeogenesis and can become sources of metabolic energy when oxidized to CO_2. The twenty AA that occur in proteins can be further broken down into two categories, essential and nonessential. The essential AA (EAA) are "indispensable" and cannot be synthesized by the animal or are synthesized at very low rates that are not sufficient enough to meet the animal's needs, especially during growth or high levels of production (NRC, 2001). These include arginine (Arg), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val). The remaining ten AA are nonessential AA (NEAA). These AA can be synthesized in sufficient amounts by the animal. These include alanine (Ala), asparagine (Asn), aspartate (Asp), glutamine (Gln), glutamate (Glu), glycine (Gly), proline (Pro), serine (Ser), taurine (Tau), and tyrosine (Tyr).

Lysine and Met have been shown repeatedly to be the first limiting EAA for milk protein synthesis under various dietary circumstances (Schwab et al., 1992). In corn-based diets containing corn-derived protein sources, Lys has proven to be the first limiting EAA (Schwab et al, 1992). Methionine has been most limiting when diets

containing high concentrations of forage were fed and soybean meal or animal derived proteins were the main source of RUP (Schwab et al., 1992). It is unknown what the ideal profile of EAA in MP should be so diets should be balanced for AA to achieve a satisfactory concentration of the most limiting EAA in MP.

Peptides in Ruminant Nutrition

Peptides are the short polymers of AA that are linked by peptide bonds. Peptides are shorter than proteins, but contain the same peptide bonds that are found in proteins (McKee and McKee, 2003). Peptides vary in length with the shortest peptides being dipeptides. Dipeptides are comprised of two AA that are joined by a single peptide bond. Oligopeptides are shorter than proteins, but contain greater than two peptides joined by peptide bonds.

Peptide Absorption

There are three different routes in which peptides can be absorbed by intestinal cells (Figure 1). Dipeptides and tripeptides are primarily absorbed through cotransport with H^+ by the transporter PepT1. Alternative routes of absorption include cell-penetrating peptides (CPP) which move across the membrane, as well as paracellular movement due to increased permeability of tight junctions (Gilbert et al., 2008).

Transport systems are able to move substrates across a cell membrane by first recognizing the substrate, then binding to the substrate, and then movement across the membrane. Transporters have been identified in endothelial cells, as well as in the apical and basolateral membranes of epithelial cells (Gilbert et al., 2008). Amino acids can be transported across the brushborder membrane of intestinal epithelial cells in their free form by a variety of transporters or as di- and tripeptides (Gilbert et al., 2008). Free AA

are carried by transporters that have differing AA specificity, whereas di- and tripeptides are carried across the membrane by the peptide transporter, PepT1. The transporter PepT1 varies from the transporters that move free AA, because PepT1 can transport all 400 di- and 8,000 tripeptides that are formed as a result of combining the 20 different dietary AA (Daniel, 2004). This transporter is also very efficient. It can transport two or three AA, while using the same amount of energy required to transport a single, free AA. Peptide transport is also much faster than the transport of free AA per unit of time (Cheng et al., 1971; Burston et al., 1972).

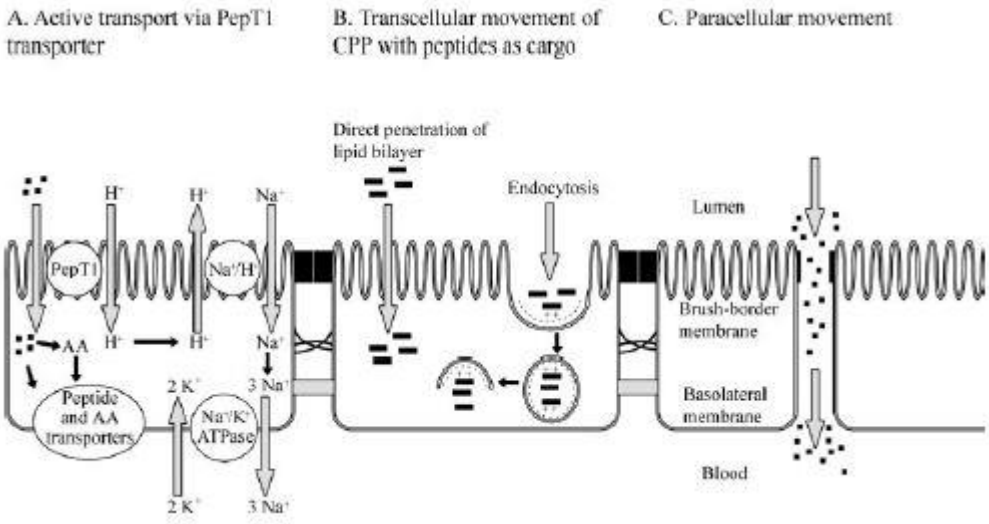

Figure 1. Potential routes of peptide uptake via the enterocytes. (A) Via cotransport with H^+ and the peptide transporter PepT1, (B) Cell-penetrating peptides, and (C) Increased permeability of tight junctions allows the uptake of peptides via the paracellular route. (Gilbert et al., 2008).

In ruminants, the flux of AA and peptides to portal drained viscera has two different routes, drainage into the mesenteric vein and non-mesenteric drain viscera. The drainage into the mesenteric vein is a result of free and peptide AA from the jejunum,

ileum, cecum, colon, and pancreas; while the non-mesenteric drain viscera comes from the flow of peptide AA from the rumen, reticulum, omasum, abomasum, duodenum, and spleen (Webb et al., 1993).

Peptide Utilization by the Mammary Gland

The AA requirements for EAA for milk protein production may not be met based upon free AA coming from the blood (Guinard and Rulquin, 1994). There have been many reports in the literature that have investigated the concept of peptides and their contribution to mammary gland metabolism and subsequent protein synthesis and secretion. Mabjeesh et al. (2005) conducted an in vivo study demonstrating that the caprine lactating mammary gland was able to use many EAA in the form of peptides for the synthesis of milk protein. Mabjeesh et al. (2005) also concluded that circulating peptides contributed to AA use for milk protein synthesis by mammary tissue with approximately 7 to 18% of methionine in casein coming from peptides. Furthermore, when the estimate of methionine derived from peptides was summed with an estimate of the contribution of free AA, total uptake of methionine by the udder was in close balance with the estimate for milk output (Mabjeesh et al., 2005). Research by Tagari et al. (2004, 2008) demonstrated that peptide-bound AA constituted an important portion of total AA flux across the portal drained viscera and also contributed to milk protein synthesis in the mammary gland.

Alternative Protein Sources

There are a few products on the market today that provide the ruminant with a source of AA, peptides, and non-protein nitrogen (NPN). By doing so, these products can replace traditional protein feeds, possibly improve rumen health, and as a result

increase microbial protein growth and flow to the small intestine (Russell et al., 1992). Because protein is the most expensive feed ingredient in many rations, there has been interest in reducing and improving ruminal protein fermentation to decrease ammonia losses (Russell et al., 1992). The rate of protein degradation varies depending upon feedstuff. Proteins in forages and soybeans are degraded rapidly by ruminal bacteria (Russell et al., 1992).

Recent advances have been made to find alternative protein supplements to replace traditional feeds such as soybean meal (Seo et al., 2008). These products widely vary, but strive to increase MP, the true protein that is digested and absorbed in the intestines (NRC, 2001). These products also improve the AA profile of the MP (NRC, 2001). These proteins and AA do not increase the dietary CP of the diet and are able to be degraded postruminally. According to Van Soest over-heating feeds can damage the protein (1994). The RUP of the protein source is increased in these feeds (Cleale et al., 1987). As a result of the heat damage, some of the AA were reduced in availability. However, the metabolism is not well understood (Nakamura et al., 1994). It can be speculated that some of the escape protein is reduced in availability due to cross-link formation between peptide chains; therefore, absorbable but not metabolizable (Hurrell and Carpenter, 1981). Among the AA, lysine is the most sensitive to heat damage resulting in its destruction and decreased availability (Mauron, 1981). Ruminally protected AA have varied production responses due to the method used to protect them from ruminal degradation (NRC, 2001).

Several companies have products on the market that are by-products of fermentation. These products contain peptides and AA that provide an ideal substrate for

microbial growth and increase the efficiency of microbial protein synthesis (Russell et al., 1992; Lean et al., 2005). Fermenten (Church and Dwight Co., Princeton, NJ) and yeast-derived microbial protein (YMP; DEMP, Alltech, Nicholasville, KY) are two examples of fermentation by-products. Penner et al. (2009) conducted a study to determine the effects of feeding Fermenten at 0 vs. 3.3% of DM (Church and Dwight Co., Princeton, NJ) with or without dietary sucrose (2.8 vs. 5.7% DM) on ruminal fermentation, apparent total-tract nutrient digestibility, and nutrient utilization. Fermenten was included by replacing canola meal and urea, and sucrose was included in the diets by replacing cracked corn grain to yield high-sugar diets. When cows were fed Fermenten with low sucrose, milk energy output increased as well as 4% FCM. It was concluded that the combination of Fermenten and supplemental sucrose did not improve the efficiency of nutrient utilization (Penner et al., 2009). It also did not support the theory that synchronizing the availability of N and fermentable energy in the rumen improves nutrient utilization with this combination of substrates (Penner et al., 2009).

Yeast-Derived Microbial Protein

Yeast-derived microbial protein is a new product on the market. It is a by-product of yeast fermentation that has been shown to be a sufficient replacement for soybean meal in dairy cow diets (Sabbia et al., 2012).

Thus far, only one study has been conducted to evaluate YMP in dairy cow diets (Sabbia et al., 2012). Sabbia et al. (2012) fed YMP at various concentrations to determine the optimal concentration at which it could replace soybean meal and provide the cow with an AA profile very close to that of MCP (Table 1). Sabbia et al. (2012) fed the YMP at 4 levels, 0, 1.14, 2.28, and 3.41% of DM. It was found that rumen ammonia

tended to decrease (*P*=0.09) as YMP linearly increased in the diet. This was attributed to the higher YMP contribution to RUP, possibly as a result of a greater rate of ruminal escape with the liquid fraction of the rumen. The substitution of soybean meal with YMP resulted in a quadratic effect on total solids percentage and a quadratic trend on milk fat percentage and yield, total solids (TS) yield, ECM and 4% FCM, possibly as a result of the YMP reaching the small intestine where it provided the cow with AA to support milk production (Sabbia et al., 2012). Thus far, no research has been conducted with YMP investigating the optimal forage concentration at which to feed this product.

Table 1. Essential AA composition of yeast-derived microbial protein (YMP), soybean meal, and microbial crude protein (MCP).

AA, % of total essential AA	YMP	Soybean meal[1]	MCP[2]
Arg	10.9	16.2	10.2
His	5.1	6.1	4.0
Ile	11.1	10.1	11.5
Leu	17.6	17.2	16.3
Lys	16.0	13.9	15.8
Met	3.6	3.2	5.2
Phe	9.6	11.6	10.2
Thr	10.0	8.7	11.7
Trp	2.9	2.8	2.7
Val	13.4	10.2	12.5
Total essential AA, % of CP	44.0	45.3	-

[1]Table 5-10 (NRC, 2001).
[2]Clark et al. (1982) and NRC (2001).
Adapted from Sabbia et al. (2012).

Importance of Forage Concentration in Dairy Cow Diets

Recommended Forage Concentration

Forage to concentrate ratios for lactating dairy cows are recommended to be between 40:60 and 60:40 (Mertens, 2009). High production cows need a ratio closer to 40:60, but if the forages are of great quality it allows for a ratio closer to 60:40 (Mertens,

2009). Within the United States, forage quality and availability varies by region. In the upper Midwest region of the United States, dairy rations typically contain 45 to 70 percent forage, using primarily corn silage and alfalfa hay (Aguerre et al., 2011).

In the literature, there is a diverse array of forage to concentrate ratios that have been investigated. Nelson et al. (1968) fed five completely pelleted rations of the following Coastal Bermuda-grass-concentrate ratios 0:100, 25:75, 50:50, 75:25, and 100:0 to cows that were between 60 and 100 DIM. There were problems encountered within the first few days of feeding a completely pelleted ration. Cows experienced cases of ruminal acidosis and compaction before they became acclimated to the diets (Nelson et al., 1968). Cows fed diets containing 75 and 100 percent forage had lower production than cows fed the other treatments. There was also a linear decrease in milk fat percentage as concentrate level in the diets increased. Aguerre et al. (2011) conducted a study in which cows were fed diets with varying forage to concentrate ratios to determine the effect on production performance, N partitioning, manure excretion and composition, and rumen fermentation pattern. The diets were 47:53, 54:46, 61:39, and 68:32 with the forage consisting of alfalfa silage and corn silage in a ratio of 1:1. There were no changes in DMI, ECM, or ECM/DMI; however, as the forage to concentrate ratio increased, milk yield and milk/DMI tended to decrease linearly (Aguerre et al., 2011). The increased forage levels also resulted in a linear increase in milk fat percentage and MUN, but a linear decrease in the concentrations of true protein, lactose, and SNF.

Effect on Rumen Kinetics

The amount of forage in the diet influences passage rate, extent of digestion, dry matter intake, and many other variables. Dry matter intake of forages may be limited by

distension resulting from the restricted flow of digesta through the gastrointestinal tract (Allen, 1996; Campling, 1970; Forbes, 1995). The reticulorumen is often the site in which distention limits DMI with high fill diets because of the presence of tension receptors (Baile and Forbes, 1974; Campling, 1970; Leek, 1986). The neutral detergent fiber (NDF) ferments slowly, and as a result it passes from the reticulorumen more slowly. Consequently, it has a much greater filling effect than other non-fibrous feed components (Allen, 1996). However, other factors, such as particle size and degradability also affect fill. Dry matter intake of low digestibility feeds is thought to be limited by physical distension (Allen, 1996). The weight of the NDF of large particles determines intake, with fluid and small particles contributing very little to fill according to Mertens (1994). When intake increases, the rate of passage from the rumen also increases (Kammes and Allen, 2012). The rumen must be able to take on more feed particles and in order to do so there must be greater passage from the rumen.

Flow from the reticulorumen is linked to particle size and specific gravity, with large particles having a longer retention time. The threshold size for sheep and cattle at maintainence, in which resistance to escape the rumen increases, is when particles are retained on a sieve of 1.18 mm (Poppi et al., 1985). Recent research has since replaced this number. The critical threshold for feed particles escaping the rumen of high producing cows has increased from 1.18 mm to approximately 4 mm (Oshita et al., 2004). Poorer quality forages have a longer retention time than forages of greater quality. The flow of particulate matter can be affected by extrinsic or intrinsic factors. Extrinsic factors are related to animal and ration characteristics (Huhtanen et al., 2006). Intrinsic factors such as particle size, rate of particle size reduction, and specific gravity are

determined by feed type, stage of maturity, leaf to stem ratio, and growth number (Lund, 2006; Kuoppala et al., 2009, 2010). Forage particles have a longer rumen retention time when compared to concentrate particles as a result of the larger particle size and lower specific gravity of the forage (Colucci et al., 1982; 1990).

The composition of the diet also affects rumen kinetics. As the forage:concentrate ratio decreases in the diet, the rumen retention time of particles increases (Huhtanen and Jaakkola, 1993). When sheep and cattle were consuming high concentrate diets, 30:70 forage to concentrate ratio, the greater concentrate diets had a slower rate of passage from the rumen (Colucci et al., 1990). The digestible energy portion of the ration also declines as intake increases and this happens at a much greater rate when the concentration portion of the diet is increased (Colucci et al., 1989). This is the result of a shorter retention time in the gastrointestinal tract (Colucci et al., 1982).

Forage type also affects retention time in the rumen. Krämer et al. (2013) found that forage type itself actually determined total-tract retention time of the forage fiber as opposed to ration composition when comparing corn and grass silages. The corn-based silage remained in the digestive tract longer than ytterbium labeled grass silage fiber (Krämer et al., 2013). When comparing rations containing two different forage:concentrate ratios (50:50 vs. 75:25) there was no difference in rumen passage kinetics of particulate matter and rumen liquid (Krämer et al., 2013).

The ratio of forage to concentrate in the diet also affects other variables. When cows were fed alfalfa hay as the only forage source at 24, 38, 58, and 80% of total DMI, organic matter digestion in the total tract increased with increasing concentrate level (Rode et al., 1985). A meta-analysis conducted by Nousiainen et al. (2009) found that

feeding concentrate in dairy rations did not improve diet organic matter digestibility due to negative associative effects. This was as a result of the decreased digestion of potentially digestible fiber (Nousiainen et al., 2009). This was in agreement with the results of Tyrrell and Moe (1975) who stated that increased concentrate feeding decreased digestibility as the percentage of concentrate increased in the diet. However, the increased diet digestibility with increased concentrate feeding found by Tyrrell and Moe (1975) was most likely an effect of lower quality forages than that of Nousiainen et al. (2009). Research has shown that as digestibility increases, DMI decreases and that as digestibility decreases, DMI increases (Blaxter et al., 1961). Feeding concentrates decreases cell wall polysaccharide digestion as a result of the reduced growth of cellulolytic microorganisms because of the reduction in pH (Hoover, 1986). Consequently, production can also be influenced. Increasing the forage to concentrate ratio from 35:65 to 55:45 on a DM basis decreased milk production. This was the result of the lower energy content of the diet, lower DMI, and lower digestion of organic matter in the total tract (Beauchemin et al., 1994, Beauchemin and Rode, 1997, Yang et al., 2001).

Conclusion

Protein nutrition is very complex and is always changing as the requirements for the dairy cow change. Due to increasing feed costs and concern for the environment, producers are looking for new ways to provide the cow with proper protein balance to meet the requirements for milk production, without excreting excess amounts of N. Forages that are of high quality are also hard to come by at times and vary by region.

Formulation of diet to meet protein requirements have changed over the years. Today, diets may be formulated based upon RDP, RUP, and MP in order to provide the cows with the proper fractions in order to maximize production. New ration formulation software are also enabling nutritionists to formulate for the cow's MP and AA needs and better predict DMI and milk production. Feed formulation models are enabling diets to be balanced for protein without feeding excess protein that would be costly and have negative effects on the environment and the cow's reproductive status.

New ingredients like YMP may provide nutritionists with other possible dietary options to meet the cow's needs, while improving N utilization and without sacrificing milk production. Incorporating YMP into the diet may provide the small intestine with an AA profile similar to that of MCP. When included in diets with greater forage to concentrate ratios, the YMP should assimilate with the liquid fraction of the rumen. Therefore, the YMP should quickly pass through the rumen before degradation by microbes is possible and provide the small intestine with an AA profile similar to that of MCP. These new products also come with new challenges at times when the forage concentrations of the diet vary, resulting in differing passage rates.

CHAPTER 2

EFFECT OF YEAST DERIVED MICROBIAL PROTEIN IN LOW AND HIGH FORAGE DIETS ON THE PERFORMANCE OF DAIRY COWS

INTRODUCTION

One of the most common challenges is to maintaining high milk production is providing sufficient amounts of RDP and RUP to meet the MP requirements for milk production and with a high utilization of nitrogen without feeding excess dietary crude protein (Agle et al., 2010a). Traditional feeds may not be able to meet the amino acid demands of high producing dairy cows. However, MCP, the protein produced by rumen microbes, has a superior AA profile compared to the AA profile in commercial feeds (Clark et al., 1992). Feeding additional RDP in the diet may fail to yield MCP when the rumen attains ammonia overflow (Satter and Slyter, 1974). By maintaining adequate RDP in the diet, a high quality source of RUP can be included to impact the profile and quantity of AA that are flowing to the small intestine, where they are able to be absorbed to enhance milk components and yield (Santos et al., 1998; Kalscheur et al., 2006).

Different strategies to provide the small intestine with greater amounts of AA without increasing the total dietary crude protein concentration in the diet need to be developed. Technology has been developed to protect AA to escape ruminal fermentation. Some of these newer technologies include slow-release urea (Taylor-Edwards, 2009), rumen protected AA (Ordway et al., 2009), oilseeds or oilseed meals that have been heat or chemically treated (NRC, 2001), and peptides (Gilbert et al., 2008). These escape proteins increase the flow of MP, the true protein that is digested and absorbed in the small intestine. These proteins and AA may not increase dietary crude protein concentration and are then able to be degraded postruminally. Sabbia et al.

(2012) substituted soybean meal with increasing levels of YMP at 0, 1.14, 2.28, and 3.41 % of DM and observed a quadratic effect on total solids percentage (P=0.02) and a quadratic trend on milk fat percentage (P=0.06) and yield (P=0.07), TS yield (P=0.08), ECM (P=0.09), and 4% FCM (P=0.08) with the inclusion rates of 2.28 and 3.41% of DM having the greatest yields and percentages. Sabbia et al. (2012) also found that rumen ammonia tended to decrease linearly as YMP increased, supporting their hypothesis that YMP may contribute to RUP by having a greater rate of ruminal escape by flowing with the liquid fraction.

Yeast-derived microbial protein was chosen for this experiment because it is a new dietary ingredient for dairy cows that can be used to replace soybean meal. However, very little research has been done to investigate the optimal forage level at which to feed this product. By directly feeding high quality YMP, it is proposed that YMP will flow at a high rate of passage with the liquid portion from the rumen (Jacques et al., 1989). By associating with the liquid portion of the rumen, degradation of YMP in the rumen would be reduced and it would pass on to the small intestine where it has the opportunity to provide the animal with high quality, readily absorbable amino acids. The YMP would then be a high quality RUP to enhance high milk production and components. This would be especially true in diets with a greater forage:concentrate ratio as the passage rate increases with increasing forage level.

The objective of this study was to determine the response of substituting soybean meal and expellers soybean meal with YMP in diets formulated with a high concentration of forage compared to a low concentration of forage on DMI, milk production and components, as well as, blood and rumen parameters of high-producing dairy cows.

MATERIALS AND METHODS

Animals and Diets

This experiment was conducted at the Dairy Research and Training Facility at South Dakota State University. All procedures were approved by the South Dakota Institutional Animal Care and Use Committee. Sixteen Holstein dairy cows (twelve multiparous and four primiparous) at 88 ± 18 days in milk (DIM) were used in a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments with four 28 d periods. Treatment diets were formulated to contain either with no YMP (NYMP) or with YMP (WYMP) (DEMP; Alltech Inc., Nicholasville, KY) at 2.25% of the diet (DM basis). Yeast-derived microbial protein replaced soybean meal, soyhulls, and mechanically extracted soybean meal in diets at low (LF) or high (HF) forage. One unit of YMP replaced 37% soybean meal, 44% mechanically extracted soybean meal, and 19% soyhulls. Cows were blocked by parity and milk production. One square of multiparous consisted of 4 ruminally-cannulated cows Diets were formulated to be isonitrogenous and isoenergetic (16.2% CP and 1.57 Mcal/kg NE_L). The ratio of alfalfa hay (33%) to corn silage (67%) was equal across all diets regardless of forage level. The LF diets were 45% forage and 55% concentrate, while the HF diets were 65% forage and 35% concentrate.

Forages were premixed in a vertical mixer and blended with concentrates in a Calan Data Ranger (American Calan Inc., Northwood, NH). Cows were individually fed for ad libitum intake once daily (0800 h) using Calan Broadbent individual animal feeders (American Calan Inc., Northdood, NH). Orts were weighed once daily and diet

offered was adjusted to ensure 10% feed refusals. Weeks 1 and 2 of each period were used for acclimation to diets and wk 3 and 4 for data collection. Cows had unlimited access to water and feed during the day, except during milking. All cows received rbST (Posilac; Monsanto, St. Louis, MO) every 14 days according to normal farm protocol.

Measurements and Sampling

Feed intakes and orts for individual cows were recorded once daily using a Calan Data Ranger (American Calan Inc., Northwood, NH). Dry matter concentration of the corn silage and alfalfa hay was determined weekly, and diets were adjusted in order to maintain the same forage: concentrate ratio throughout the experiment.

Samples of alfalfa hay, corn silage, concentrate mixes, and total mixed ration (TMR) of each treatment were collected twice during wk 3 and 4 of each period, frozen and stored at -20°C until compositing. Samples of individual ingredients were collected from the feed mill each time that the concentrate mixes were mixed and delivered. Individual feed ingredients were then equally composited into one composite at the end of the study. Additional samples of the TMR treatments were taken once during wk 3 and 4 to determine particle size and physically effective fiber using the Penn State Particle Separator (PSPS) and Z Box.

Ruminal fluid was sampled from each of the cannulated cows on d 27 of each period over 9 time points. Samples were taken just prior to feeding, and, 2, 4, 6, 8, 10, 12, 16, and 24 h after feeding. Ruminal fluid samples were collected using a 50 mL syringe and a stainless steel suction device to collect 10 mL from 5 separate locations throughout the rumen for a total of 50 mL of ruminal fluid. Locations sampled were the cranial and caudal rumen mat, cranial sac, ventral sac, and caudal blind sac of the rumen.

Samples were immediately measured for pH, and 10 mL aliquots of rumen fluid were placed in scintillation vials. One vial contained 200 µL of 50% (vol/vol) sulfuric acid and the other contained 2 mL of 25% (wt/vol) metaphosphoric acid. Samples were frozen and stored at -20°C until analyzed for ammonia and volatile fatty acid (VFA) analysis.

Blood was collected by venipuncture of the coccygeal vein approximately 3 h after feeding on two consecutive days during wk 4 of each period from each cow. Blood was drawn into a 10-mL Vacutainer® tubes containing lithium heparin for AA and PUN, and a 7-mL Vacutainer® tube containing sodium fluoride potassium oxalate for glucose (Becton, Dickinson, and Company, Franklin Lakes, NJ). Blood was also collected from the mammary vein and drawn into 10-mL Vacutainer® tubes containing lithium heparin (Becton, Dickinson, and Company, Franklin Lakes, NJ). Blood samples were centrifuged at 2,000 rpm for 20 min at 5°C (CR412 centrifuge; Jouan Inc., Winchester, VA) and plasma was collected and frozen until analysis.

Cows were milked 3 times daily (0600, 1400, an 2100 h) in a double-8 parallel milking parlor (DeLaval, Tumba, Sweden) equipped with automatic cow identification, individual production recording, and automated detaching milking units. Milk from individual cows was sampled at each milking on 2 consecutive days during wk 3 and 4 for milk composition analysis.

Body weights (BW) were recorded on 3 consecutive days at the beginning and end of each period. Body condition score (BCS: 1-5 scale; Wildman et al. (1982)) was assessed by 3 independent observers at the beginning and end of each period.

Laboratory Analysis

All feed and TMR samples were composited by period, with the exception of the individual ingredients which were composited into one sample, dried for 48 h at 55°C in a Despatch oven (style V-23; DespatchOven Co., Minneapolis, MN), ground through a 4-mm screen using a Wiley mill (model 3; Arthur H. Thomas Co., Philadelphia, PA), and then further ground through a 1-mm screen (Brinkman ultracentrifuge mill, Brinkman Industries Co., Westbury, NY). Subsamples of feed composites were dried at 105°C for 3 h for DM determination (Shreve, 2006). Composition analysis was determined at Alltech Laboratories (Alltech Inc., Brookings, SD), NDF was analyzed with sodium sulfite and α-amylase (Van Soest et al., 1991) and ADF (Robertson and Van Soest, 1981) sequentially using an ANKOM fiber analyzer (ANKOM Technology Corp., Fairport, NY). Lignin was determined after the sample had been run for acid detergent fiber (Van Soest, 1963). Crude protein was determined using an Elementar Rapid N Cube (Elementar, Hanau, Germany; Method 968.06; AOAC, 2006). Ether extract was determined using an ANKOM extractor with diethyl ether as the solvent (920:39; AOAC, 2006). Neutral detergent insoluble N (NDIN) was determined using an ANKOM fiber analyzer (ANKOM Technology Corp., Fairport, NY) without sodium sulfite, after digestion the residue was analyzed for CP using an Elementar Rapid N Cube (Elementar, Hanau, Germany; Licitra et al., 1996). Acid detergent insoluble nitrogen (ADIN) was determined using an ANKOM fiber analyzer (ANKOM Technology Corp., Fairport, NY), followed by CP analysis of the residue using a Rapid N Cube (Elementar, Hanau, Germany) after digestion (Licitra et al., 1996). Soluble protein concentration of the forages, concentrate mixes, and individual ingredients was determined using a Borate

buffer (Licitra et al., 1996). Starch of the forages, concentrate mixes, and individual ingredients was determined according to Hall (2009).

Composites of alfalfa hay, corn silage, concentrate mixes, and individual ingredients were sent to DairyLand Laboratories Inc. (Arcadia, WI) and analyzed for the following minerals: Ca, P, K, Mg, and S according to AOAC procedures (method 953.01; AOAC 2002). Composites of alfalfa hay, corn silage, concentrate mixes, and individual ingredients were also sent to the University of Missouri Agriculture Experiment Station Chemical Laboratories (Colombia, MO) for a complete AA profile analysis (method 982.30; AOAC, 2006). Particle size distribution of the diets was determined by the 4-screen PSPS (Kononoff et al., 2003) using fresh feed samples. Physically effective fiber was determined using the Z Box on fresh feed samples (W. H. Miner Agricultural Research Institute, Chazy, NY).

Rumen fluid samples conserved with metaphosphoric acid were analyzed for VFA concentration. Samples were thawed and centrifuged at 15,000 × g for 15 minutes. Ethyl butyrate was used as the internal standard. Analyses were conducted on a 7890A gas chromatograph equipped with a 7693 auto sampler (Agilent Technologies, Hewlett Packard, Palo Alto, CA). Peaks were separated using a capillary column 25m × 150µm × 0.25µm (Agilent CP 7686). Oven programming was 90°C, 15°C/min to 180°C, 90°C/min to 250°C hold for 2 minutes. FID was maintained at 270°C, inlet with a 30:1 split. Ammonia-N concentration was determined in rumen fluid samples conserved with sulfuric acid. Rumen sub-samples were centrifuged at 10,000 ×g for 10 minutes and analyzed for ammonia-N concentrations as described by Weatherburn (1967).

Plasma glucose was determined by glucose oxidase reaction (Trinder, 1969) with a glucose kit (glucose kit, code 301001-081, Pointe Scientific, Inc., Canton, MI). Plasma was composited by cow by period and blood glucose preparations were read in a microplate reader (Cary 50 MPR, Varian Inc., Lake Forest, CA). Plasma was composited by cow by period and shipped to the University of Missouri Agriculture Experiment Station Chemical Laboratories (Colombia, MO) to determine plasma free amino acid concentrations (Deyl et al., 1986; Fekkes, 1996). Arteriovenous difference was calculated as: arterial plasma concentration – venous plasma concentration for each of specific AA. Extraction efficiency was calculated as follows: Extraction efficiency = AV difference/arterial concentration × 100. Amino acids were classified into EAA and NEAA based on their importance for milk protein synthesis (Clark et al., 1978). The EAA were His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val; NEAA were Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr; and branched-chain amino acids (BCAA) were Ile, Leu, and Val. Total amino acids (TAA) was calculated as the sum of EAA and NEAA.

Milk samples were sent to Heart of American DHIA Laboratory (Manhattan, KS) for composition analysis. Fat, protein, lactose, and solid not fat (SNF) were analyzed via mid-infrared spectroscopy (AOAC, 2006; Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN), and milk urea nitrogen (MUN) was determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments, Chaska, MN). Somatic cells were counted using laser technology (Soma Count 500, Bentley Instruments, Chaska, MN).

Data Analysis

All data were analyzed using the MIXED procedure of SAS (SAS, 2001). Weekly means of DMI and milk yield during the final 2 wk of each period were used for statistical analysis. Means for milk composition were determined from individual samples taken at each milking twice weekly during wks 3 and 4. Plasma metabolites were from samples collected on two consecutive days during wk 4, BW from three consecutive days at the beginning and end of each period, and BCS at the beginning and end of each period. These data were analyzed using the following fitted model:

$$Y_{ijklm} = \mu + Forage_i + YMP_j + (Forage_i \times YMP_j) + (Forage_i \times S_m) + (YMPj \times S_m) + (Forage_i \times YMP_j \times S_m) + P_k + C_l(S_m) + S_m + \varepsilon_{ijklm},$$

where Y_{ijklm} is the dependent variable, μ is the overall mean, $Forage_i$ is the effect of forage level i (i=1 to 2), YMP_j is the effect of YMP j (j=1 to 2), $(Forage_i \times YMP_j)$ is the effect of the interaction of forage level i and YMP level j, P_k is the effect of period k (k=1 to 4), $C_l(S_m)$ is the effect of cow l (l=1 to 4) nested within square m, S_m is the effect of square m (m=1 to 4), and ε_{ijklm} is the residual error. The experimental design used cow as experimental unit and cow(square) as the random variable. Main effects were tested against square but were found to be not significant (P>0.05) and therefore are not reported.

A repeated measures model was used to evaluate ruminal parameters (pH, NH_3, and VFA concentrations), using the following model:

$$Y_{ijkn} = \mu + Forage_i + YMP_j + (Forage_i \times YMP_j) + P_k + \varepsilon_{ijk} + H_n + (H_n \times Forage_i \times YMP_j) + \omega_{ijkn},$$

where Yijkn is the dependent variable, μ is the overall mean, $Forage_i$ is the effect of forage level i (i=1 to 2), YMP_j is the effect of YMP j (j=1 to 2), $(Forage_i \times YMP_j)$ is the

effect of the interaction of forage level i and YMP level j, P_k is the effect of period k (k=1 to 4), ε_{ijk} is the whole plot error, Hn is the effect of time n (n=1 to 9), ($H_n \times Forage_i \times YMP_j$) is the interaction between time n, Forage i, and YMP j, and ω_{ijkn} is the sub-plot error.

Interactions that were deemed insignificant ($P \geq 0.05$) were removed from the models. Significance was declared at $P \leq 0.05$, and tendencies were discussed at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Nutrient Content of the Diets

Ingredient composition of the experimental diets is shown in Table 2. Diets were formulated to contain the same ratio of corn silage to alfalfa hay regardless of forage inclusion (Table 3). Soybean meal, mechanically expelled soybean meal, and soybean hulls replaced YMP in the NYMP diets. Diets were formulated to be isoenergetic (1.57 Mcal/kg NE_L) and isonitrogenous (16.2% CP) and the dietary CP concentrations remained fairly constant across treatments; however, the analysis for CP was slightly lower than formulated and the resulting NE_L calculation was slightly greater (Table 4). Analyzed dietary treatments varied slightly from formulated values in NDF, ADF, lignin, starch, ether extract, and ash; however, these nutrients were similar across treatments (Table 4). The analyzed nutrient composition of the individual ingredients on which the analyzed nutrient composition of the experimental diets was based is presented in Table 5.

Diets were formulated to contain either 45 or 65% forage and between 32 and 33% NDF to support high production of milk solids without decreasing milk yield or

DMI (Dado and Allen, 1995). The diets containing the greater forage inclusion could allow for greater fluid passage rates (Colucci et al., 1990; Rotger et al., 2005). Therefore, if the YMP was diluted in the liquid fraction of the rumen as hypothesized, a larger amount of small peptides, oligopeptides, and soluble protein should escape ruminal fermentation and reach the small intestine to support milk production at the high forage inclusion.

The EAA profile in relation to CP % (Table 6) demonstrated that YMP actually had an AA profile similar to that of soybean proteins, but had greater concentrations of Lys, Met, and Trp than soybean meal. The experimental diets were similar in AA concentration across forage level (LF vs. HF) regardless of the addition of YMP (Table 7). There were slight increases in the concentration of Lys and Met due to the addition of YMP; however, numerically this difference was very small and may not have been large enough to elicit a response. Sabbia et al. (2012) found that YMP had an EAA profile similar to that of MCP, and a better composition of Met and Lys than soybean meal, the most limiting AA in milk production (Schwab et al., 1992; Sabbia et al., 2012).

Particle size of the diets measured with the PSPS (Table 8) showed differences in retention on the screens between forage levels ($P<0.001$). The LF diets had more retention on the 1.8 mm screen and bottom pan than the HF diets (31 vs. 23.5% for the LF and HF, respectively; $P<0.001$). These values may be greater as a result of mixing and sampling error. The increased proportion of particles on the 19 mm screen with increasing forage:concentrate was expected because only the forage portion of the diet contributed large particles. The increased proportion of particles on the 19 mm screen with increasing forage:concentrate was consistent with Yang and Beauchemin (2009).

There were no differences in retention on the sieves due to YMP or the interaction of F × YMP. This was as expected due to the nature of the YMP. It was not expected to be retained upon the sieves, nor interact with the forage to cause added retention. Sabbia et al. (2012) utilized similar dietary ingredients with a forage:concentrate ratio of approximately 60:40. The retention on the upper screen and bottom pan in the current experiment were greater than those reported in Sabbia et al. (2012). However, the 8 mm screen had the greatest retention, which is consistent with Sabbia et al. (2012) although the values differ.

The pef differed between forage level (Table 8) with the HF diets having greater pef than the LF diets (P=0.02). There were no differences in pef due to YMP or the interaction of F × YMP (P=0.64 and P=0.45, respectively). The increased pef with HF diets was expected because only the forage portion of the diet contributed to large particles. The YMP was not expected to cause any pef differences.

Production Measures

There was no effect of forage concentration or YMP on BW or BW change (Table 9). There was an effect of forage on BCS (P=0.05) and BCS change (P=0.05) with cows on the LF diets having a greater BCS than those fed the HF diets (3.09 and 3.04 respectively). Although significant, this resulted in very minimal and almost indiscernible differences in BCS. There was no F × YMP interaction for BW or BCS.

Dry matter intake was affected by forage level (P<0.001). Cows fed the LF diets consumed approximately 1.6 kg/d more DM than cows fed the HF diets (Table 9). These cows also had the greatest BCS and BCS change reflecting their increased DMI. It is likely that these cows had more rumen fill and appeared to be putting on more weight to

the individual observers assigning BCS. These results are similar to those found by Sabbia et al. (2012), who found that cows fed 0 or 2.24% DM YMP had the lowest intakes. This study had a forage:concentrate of approximately 60:40 which is comparable to the HF diets in the current study. In the current study, YMP had no effect on DMI (P=0.35).

Dry matter intake is a function of meal size and meal frequency which are determined by animal and dietary factors affecting hunger and satiety (Allen, 2000). A greater initial meal size results in the production of sensory stimuli that may alter the threshold by which the brain satiety centers trigger meal cessation by distention stimuli (Allen, 1996). This occurs for the first meal after feeding but not for subsequent meals, resulting in a lower daily DMI (Allen, 1996). Sabbia et al. (2012) speculated that cows receiving 2.25% of DM YMP possibly had a large, early meal, resulting in an earlier peak in total ruminal VFA concentration, an earlier drop in ruminal pH, and a greater peak of ruminal ammonia concentration. It was hypothesized that this was the reason for the lower DMI. In the current study, cows fed the HF diets had a lower DMI than those fed the LF diets. This is consistent with the results of previous studies that fed cows diets differing in forage:concentrate (Yang and Beauchemin, 2007). However, the pH (Figure 1), ruminal ammonia (Figure 2), and total VFA concentration (Figure 3) do not parallel that of Sabbia et al. (2012). The HF treatments do have a greater peak of ruminal ammonia (Figure 3) but this is not reflected in the ruminal pH and total ruminal VFA. Therefore, the decreased DMI may possibly be the result of distension as a result of the restricted flow of digesta through the gastrointestinal tract (Allen, 1996).

Reducing the concentration of forage in the diets affected milk production (Table 9). Milk production was greater for cows fed the LF diets than for those fed the HF diets (40.1 kg/d and 37.8 kg/d respectively). This decrease in milk production was consistent with lower DMI for cows fed the HF diet compared to cows fed the LF diet. Li et al. (2012) fed diets containing 35:65 and 60:40 forage:concentrate and reported that cows fed diets lower in forage produced more milk (28.8 vs. 25.9 kg/d, respectively). Agle et al. (2010) fed a low (52% of DM) and a high concentrate (72% of DM) diet and observed increased milk production for cows fed the high concentrate diet (33.2 vs. 36 kg/d, respectively). Yang and Beauchemin (2007) observed similar results when feeding cows one of two forage:concentrate levels (35:65 vs. 60:40). In that study the decrease in milk production for cows fed diets high in forage was attributed to decreased DMI and lower intake of digestible organic matter in the total tract. In the current experiment, digestibility was not measured but could play a role in the decreased milk production for cows that were fed the HF diet compared to the LF diet. There were no F × YMP interactions for milk yield. The addition of YMP also did not affect milk yield.

Milk fat percentage was affected by forage level; however, fat yield was not (Table 9). Cows fed the HF diets had a greater percentage of fat in their milk compared to those fed the LF diets (P=0.04). The differences in milk fat between forage levels could reflect differences in ruminal digestion. In the current study there was a tendency for F × YMP interaction on ruminal pH and propionate concentration. Agle et al. (2010) and Li et al. (2012) reported similar results. Similar results were also observed by Yang and Beauchemin (2007). They also attributed the differences in milk fat percentage to

increased fiber digestion and utilization as a result improved ruminal fermentation with an increased proportion of forage in the diet.

Cows fed WYMP tended to have lower fat yield than cows fed NYMP; however. When compared to the results of Sabbia et al. (2012), cows on the current experiment fed WYMP actually had similar fat yields to the cows fed YMP at 2.25% of DM. It appears that YMP played a role in ruminal fermentation. It is not known how and this warrants further investigation. There was no F × YMP interaction for fat percentage or yield.

Protein percentage was not different between treatments. However, cows fed the LF diets had greater protein yield compared to cows fed the HF diets ($P<0.001$). The concentration and yield of milk protein can be influenced by the profile of AA in MP, by the amount of surplus protein, and by fermentable carbohydrates in the diet (NRC, 2001). Literature suggests, small or many times, no increases in milk yield and protein content when dietary protein, AA, or energy are supplemented (Bequette et al., 1998). This is especially true when energy and protein are not limiting production (Brun-Lafleur et al., 2010). Therefore, it appears that knowledge in this area is incomplete in discerning how dietary nutrients affect or limit milk protein synthesis. In the current experiment, it should be noted that milk protein percentage (3.24%) could be considered high explaining the possible lack of response to treatments. There was no F × YMP interaction of protein percentage or yield.

The experimental diets resulted in differing total solids percentages as well as yields; however, there were no differences in lactose (Table 9). There was also an effect of YMP inclusion on total solids percentage. Cows fed NYMP ($P=0.10$) tended to have greater lactose percentage than those fed WYMP. Cows fed NYMP had greater ($P=0.05$)

total solids percentage than cows fed WYMP; however due to the increased production of cows fed the LFWYMP treatment these differences as a result of YMP were not reflected in total solids yield. There was no F × YMP interaction of lactose or total solids percentage or yield.

A forage effect on MUN was observed. Cows fed the HF diets had a greater (P<0.001) MUN than cows fed the LF diets. This is consistent with the cows fed the HF diets having greater ruminal ammonia concentrations as well as greater PUN levels than cows fed the LF diets. The elevated MUN levels in the cows fed the HF diets is a result of the greater ammonia concentration of cows fed the HF diets. When Sabbia et al. (2012) fed YMP at 0, 1.14, 2.25, and 3.41% of DM a quadratic effect on MUN was observed. In the current experiment, however, there was no effect of YMP on MUN. There was also no F × YMP interaction of MUN.

Cows fed WYMP were less efficient than the cows that were fed the NYMP. This was a result of the cows fed the WYMP eating just as much as those that were fed the NYMP, but producing less milk. It is difficult to interpret the reason for this response. It may be that the concentration of YMP in the diets was too great. Sabbia et al. (2012) found that YMP elicited a response at 1.14% of DM. It is possible that the current experiment over fed YMP, yet this cannot be explained and warrants further investigation. There was no F × YMP interaction of ECM or ECM/DMI.

There were no differences in SCS because of forage level or YMP. Sabbia et al. (2012) did not observe any differences in somatic cell count and in the current experiment neither forage level nor YMP were expected to yield differences in SCS.

Ruminal Fluid Analysis

An interaction ($P<0.01$) of forage and YMP was observed for propionate concentration, acetate and propionate proportion, and acetate:propionate ratio, as well as a tendency ($P=0.09$) for an interaction of forage and YMP for ruminal pH (Table 10). A forage effect was observed for ruminal ammonia, isobutyrate concentration, butyrate concentration, isovalerate concentration, as well as proportions of isobutyrate, butyrate, and isovalerate with cows on HF diets having greater concentrations and proportions of these measures. There was an effect of YMP on butyrate proportion with WYMP cows having a greater proportion. The reason for this is unknown.

Sabbia et al. (2012) reported a tendency for a linear decrease in ruminal ammonia as the concentration of YMP in the diet increased. There were no differences in ruminal ammonia due to YMP in this experiment; however, HF diets had a greater concentration of ammonia. Yang and Beauchemin (2009) reported an increase in propionate concentration with increasing forage:concentrate. These researchers also found increased concentrations of isobutyrate, butyrate, and isovalerate as the forage concentrate of the diet increased which is inconsistent with these results. It was expected that cows fed the HF diets would have greater concentrations of acetate because the increased forage concentration of the diet would be expected to ferment to greater acetate within the rumen. Likewise, the LF diets were expected to have greater concentrations of propionate. In this experiment there was a F × YMP interaction on ruminal proportions of acetate and propionate (Table 10). The reason for this is unknown. However, YMP seems to have had an effect on microbial activity in the rumen. Yang and Beauchemin (2009) found that increasing the forage:concentrate from 35:65 to 60:40 decreased total VFA concentration, resulting in increased ruminal pH. Increasing the dietary forage level

would have reduced acid production due to less starch available for fermentation (Yang and Beauchemin, 2007). The greater ruminal pH would favor fibrolytic activity and increase the acetate:propionate ratio. In the present study, there were no changes in pH and total VFA concentrations due to the concentration of forage and concentrate level in the diets.

There were no interactions of F × Time, YMP × Time, or F × YMP × Time on ruminal pH, ammonia, and total VFA (Figures 2, 3, and 4, respectively). However, there was an effect of time on ruminal pH (Figure 2). At approximately 8 to 10 hours post-feeding cows had the greatest ruminal pH. There was an effect of time on ruminal ammonia and total VFA concentration (Figures 3 and 4, respectively). Changes in ruminal pH, ammonia, and VFA concentrations over time were most likely the result of the frequency and length of meals of the cows. Sabbia et al. (2012) speculated that peaks in the ruminal ammonia and total VFA concentration patterns of cows fed 3.41% YMP of DM could be explained by an increased supply of AA that increased the rate of clearance of metabolic fuels from the blood, resulting in hunger and reducing the inter-meal interval. The HF diets also yielded peaks in ruminal ammonia over time (Figure 2), but when looking at plasma AA data, the HF diets generally had lesser circulating plasma AA.

Blood Analysis

There were no differences in plasma glucose concentrations between forage or YMP levels (Table 11). Sabbia et al. (2012) found slightly greater plasma glucose concentrations when cows were fed the same inclusion of YMP as this experiment. Increasing the inclusion rate of YMP in the diet resulted in a tendency for a cubic effect

on plasma glucose concentrations (Sabbia et al., 2012). When looking at what is considered the normal range for bovine plasma glucose levels, the concentrations of the current experiment fall within the normal range (Bergman, 1971).

There was a forage effect on arterial and venous PUN concentrations. Cows fed the HF diets had greater PUN concentrations. Arterial and venous PUN levels were consistent with values typically reported in the literature. Kauffman and St-Pierre (2001) fed diets containing approximately 16.7% CP and found that PUN was approximately 15.6 mg/dL. The PUN concentrations did follow those of MUN with the HF diets having both greater PUN and MUN concentrations.

There was a forage effect on the EAA including Ile, Leu, Lys, Met, Thr, and Val (Table 12). Cows fed the LF diets had greater concentrations of these circulating arterial plasma AA than cows fed the HF diets ($P<0.05$). Greater arterial concentrations of Ile, Lys, Trp, and Val were found in cows fed the NYMP diets compared to cows fed WYMP diets. There was a tendency for cows fed NYMP diets to have greater arterial concentrations of Arg, His, Leu, and Thr. There was a tendency for a forage and YMP interaction for circulating arterial Tau concentratons ($P=0.07$). There was a forage effect on circulating arterial plasma Ala, Asn, Gly, Pro, and Ser with cows fed the LF diets having greater concentrations for all but Ala in which cows fed HF diets had greater circulating concentrations. There tended to be greater circulating arterial Tyr in cows fed the LF diets ($P=0.07$). A YMP effect was observed for circulating arterial Asp, in which cows fed NYMP had greater concentrations of Asp than cows fed WYMP. Arterial plasma concentrations of EAA, NEAA, BCAA, and TAA were greater for cows fed LF diets than for cows fed HF diets ($P<0.01$).

There were no forage and YMP interactions for any of the venous circulating AA. There were differences in circulating venous AA concentrations due to the effect of forage as well as YMP (Table 13). A forage effect on circulating concentrations of Ile and Val was observed with cows fed LF diets having greater concentrations of these AA than cows fed HF diets. Cows fed NYMP had greater circulating venous concentrations of Arg, His, Ile, Lys, and Val than cows fed WYMP. These cows also tended to have greater concentrations of circulating venous Thr than cows fed WYMP (P=0.06). There was a forage effect on circulating venous plasma Gly with cows fed LF diets having greater concentrations than cows fed HF diets. A tendency for LF diets to have greater circulating venous concentrations of Ala was also observed. There was a tendency for an interaction of forage and YMP on circulating venous Tau concentrations. Forage level affected circulating venous plasma concentrations of EAA, NEAA, BCAA, and TAA with LF diets having greater concentrations than cows fed HF diets. There were also YMP effects on EAA and BCAA with NYMP diets having greater concentrations of these circulating venous AA than WYMP diets. A tendency for WYMP diets to have lesser circulating venous TAA than NYMP diets was also observed.

The arterial and venous plasma AA concentrations parallel each other. Arterial differences were often reflected within the venous concentrations as well. These plasma concentrations were consistent with other values found in the literature (Kung et al., 1984; Yeo et al., 2003; Mjoun et al., 2010). According to the literature, the limiting AA for milk production are Lys and Met and both circulating arterial and venous concentrations of Lys were greater for cows fed NYMP than for cows fed WYMP (P<0.01 and P=0.01 respectively). Despite forage and YMP effects on arterial Lys

concentrations, no differences were observed in milk protein percentage (presented later in the paper). The mammary gland was able to maintain milk protein synthesis most likely as a result of the consistent extraction efficiency of Lys across treatments.

The concentration of forage included in the diets affected arteriovenous differences of AA (Table 14). The arteriovenous differences of Arg, Ile, Leu, Lys, Met, Phe, Thr, and TEAA were greater in cows fed LF diets compared to cows fed HF diets. There was a tendency for LF diets to have greater arteriovenous differences of Val than HF. Tendencies for cows fed WYMP compared to NYMP to have lesser arteriovenous differences of Lys and Trp were also observed, possibly explaining the decreased milk production in these treatments.

Cows fed LF diets had greater AV differences of Asn, Ser, and Tyr than cows fed HF diets. A tendency for cows fed WYMP to have lower arteriovenous differences of Asp was observed compared to cows fed NYMP. There was a forage effect on EAA, NEAA, BCAA, and TAA. Cows fed LF diets had greater arteriovenous differences than cows fed HF diets. This was most likely a direct effect of the cows fed LF diets having greater dietary concentrations of these AA than cows fed the HF diets. There is very limited research on circulating arterial and venous plasma AA in which to compare the results from the current experiment. A few researchers have investigated feeding diets differing in forage:concentrate and the AA composition of the diets (Yang and Beauchemin, 2004; Li et al., 2012). These researchers, however, reported duodenal AA concentrations and not plasma AA. Yang and Beauchemin (2004) fed diets in which the forage was comprised of alfalfa silage, barley silage, and alfalfa hay. The forage:concentrate was 35:65 and 55:45. Increasing the forage proportion of the diet had

little effect on the flows of AA to the duodenum. Li et al. (2012) fed diets containing low and high forage inclusion rates (35:63 vs. 60:40, respectively) in which alfalfa silage was the primary forage source. The low forage diets had greater duodenal flows of all AA, with the exception of Trp, which was not affected by dietary forage concentration (Li et al., 2012). Hussein et al. (1995) fed beef steers either 70 or 30% forage as % of DM. The forage was comprised of solely corn silage. When looking at the concentrations of AA in the ruminal bacteria of these steers, the steers fed 30% forage had greater concentrations of AA with the exception of the concentrations of Ile, Leu, Lys, and Phe, which were not affected by forage inclusion (Hussein et al., 1995).

Extraction efficiency of Arg was greater for cows fed LF diets than those fed HF diets (Table 15). There was also a tendency for the interaction of forage and YMP for the extraction efficiency of Met. Cows fed LF diets had a greater extraction efficiency of Gln than cows fed HF diets. A tendency for a greater extraction efficiency for Asp for the LF diets compared to the HF diets was also observed. There were no interactions of forage and YMP for the extraction efficiency of any of the AA.

Conclusion

The addition of YMP, as well as dietary forage concentration, affected ruminal fermentation. According to the conditions of this study, the inclusion level of forage, as well as YMP inclusion appear to affect ruminal fermentation. These changes in ruminal fermentation however, were not reflected in production.

The substitution of soybean meal by YMP affected the arterial and venous plasma concentrations of EAA, especially the limiting AA Lys. However, these changes were not reflected in the extraction efficiency of Lys.

Production measures were affected by forage amount and substitution of YMP for soybean products, but there were no F × YMP interactions. Cows fed the LF diets had greater DMI than those fed the HF diets, which resulted in cows fed LF having greater milk production than cows fed HF diets. Energy corrected milk was greater for cows fed the LF diets compared to the HF diets; however, cows fed the LF diets had lower milk fat percentage in their milk. Feed efficiency was greater for cows fed NYMP than for cows fed WYMP. As a result, YMP does not appear to improve the production of high producing dairy cows fed diets formulated at either low or high forage concentrations.

Table 2. Ingredient composition of the experimental diets.[1]

	LF		HF	
Item, % of DM	NYMP	WYMP	NYMP	WYMP
Corn silage	30.00	30.00	43.35	43.35
Alfalfa hay	15.00	15.00	21.65	21.65
Dried ground corn	17.37	17.37	12.92	12.92
Dried distillers grains with solubles	3.07	3.07	3.97	3.97
Corn gluten feed	8.01	8.01	1.91	1.91
Soybean meal (46% CP)	4.50	3.67	5.40	4.57
Expellers soybean meal[2]	4.66	3.67	5.56	4.57
Soybean hulls	14.13	13.71	1.70	1.27
Rumen inert fat[3]	1.16	1.16	1.57	1.57
YMP[4]	0	2.25	0	2.25
Limestone	0.90	0.90	0.64	0.64
Sodium bicarbonate	0.45	0.45	0.45	0.45
Salt	0.26	0.26	0.26	0.26
Vitamin premix[5]	0.15	0.15	0.15	0.15
Dicalcium phosphate	0.15	0.15	0.30	0.30
Magnesium oxide	0.15	0.15	0.15	0.15
Vitamin E[6]	0.04	0.04	0.04	0.04

[1]LF= low forage; HF = high forage; NYMP= no yeast-derived microbial protein; WYMP= with yeast-derived microbial protein.
[2]AMINOPLUS (Ag Processing, Inc., Omaha, NE).
[3]Energy Booster 100 (Milk Specialties Global, Eden Prairie, MN).
[4]DEMP (Alltech Inc., Nicholasville, KY).
[5]Contained: 21.5% Ca; 320 mg/kg of Co; 7500 mg/kg of Cu; 500 mg/kg of I; 5000 mg/kg of Fe; 34,844 mg/kg of Mn; 165 mg/kg of Se; 34,844 mg/kg of Zn; 3,740,000 IU/kg of Vitamin A; 935,000 of IU/kg of Vitamin D; 12,155 IU/kg of Vitamin E (Hubbard, Mankato, MN)
[6]Contained: 20,000 IU/kg.

Table 3. Formulated nutrient composition of the experimental diets.[1]

	LF		HF	
Item, % of DM	NYMP	WYMP	NYMP	WYMP
DM, % of diet	60.1	60.2	52.5	52.5
CP	16.2	16.2	16.2	16.2
RDP[2]	9.9	10.0	10.1	10.1
RUP[2]	6.3	6.2	6.2	6.2
NE_L, Mcal/kg of DM[2]	1.57	1.57	1.57	1.57
NDF	33.9	33.5	32.9	32.5
ADF	21.0	20.7	20.3	20.0
Starch[3]	25.7	25.7	25.7	25.8
NFC[4]	41.8	42.2	42.3	42.8
Ether extract	3.8	3.8	4.0	4.0
Ash	6.68	6.80	6.94	6.93
Ca	0.88	0.87	0.89	0.88
P	0.38	0.39	0.38	0.38

[1]LF= low forage; HF = high forage; NYMP= no yeast-derived microbial protein; WYMP= with yeast-derived microbial protein.
[2]Estimated from NRC (2001) according to nutrient composition of the ingredients.
[3]Estimated from CPM Version 3 Software.
[4]NFC= 100-(%NDF + %CP + %EE + % ash).

Table 4. Analyzed nutrient composition of the experimental diets based on individual ingredients.[1]

	LF		HF	
Item, % of DM	NYMP	WYMP	NYMP	WYMP
DM, % of diet	62.3	62.4	55.1	55.1
CP	16.1	16.0	16.0	16.0
RDP[2]	9.9	9.9	9.9	9.9
RUP[2]	6.2	6.2	6.2	6.2
Soluble protein, % of CP	44.8	43.9	44.7	43.8
NDIN	3.08	3.10	3.02	3.11
ADIN	0.73	0.70	0.73	0.72
NE_L, Mcal/kg[2]	1.59	1.59	1.59	1.59
NDF	33.7	32.2	32.1	31.6
ForageNDF	18.5	18.5	26.8	26.8
ADF	20.0	18.9	18.8	18.5
Lignin	2.23	2.56	2.66	2.65
Starch	24.0	24.3	24.4	24.5
NFC[3]	41.7	43.3	43.3	43.9
Ether extract	3.15	3.20	3.22	3.22
Ash	5.31	5.32	5.37	5.32
Ca	0.88	0.87	0.87	0.86
P	0.38	0.40	0.37	0.39
Mg	0.39	0.39	0.39	0.38
K	1.14	1.11	1.08	1.05
S	0.25	0.25	0.21	0.21

[1]LF= low forage; HF = high forage; NYMP= no yeast-derived microbial protein; WYMP= with yeast-derived microbial protein.
[2]Estimated from NRC (2001) according to the analyzed nutrient composition of the ingredients.
[3]NFC= 100-(%NDF + %CP + %EE + % ash).

Table 5. Analyzed nutrient composition of the individual ingredients used in the lactation study.

Item, % of DM	Alfalfa hay	Corn silage	YMP[1]	Dried ground corn	Dried distillers grains with solubles	Corn gluten feed	Soybean meal, 46%	Expellers soybean meal[2]	Soyhulls
DM	88.6	36.9	93.11	86.1	89.1	87.8	88.1	87.9	90.2
CP	18.5	7.45	44.05	8.99	30.7	26.8	52.6	51.1	11.8
Soluble protein, % of CP	50.6	55.2	-	21.5	18.0	69.1	45.5	29.8	52.3
NDIN	4.76	1.00	-	1.32	7.44	2.86	10.2	7.48	4.03
ADIN	1.72	0.36	-	0.13	2.38	0.36	1.26	1.03	0.95
NDF	47.8	37.9	-	8.92	31.7	27.0	7.96	15.8	66.5
ADF	36.7	19.9	-	2.63	9.39	7.47	4.18	6.94	47.2
Lignin	8.88	1.35	-	0.46	1.23	0.31	0	0.03	2.47
Starch	0.64	33.3	2.85	72.3	3.07	13.7	1.17	1.02	0.03
Ether extract	1.80	3.48	1.62	3.71	15.1	3.40	1.43	1.09	2.39
Ash	9.13	4.53	4.59	0.98	4.78	10.1	5.99	7.25	5.29
Ca	1.53	0.27	0.09	0.03	0.05	0.17	0.43	0.62	0.64
P	0.26	0.20	1.17	0.25	0.95	1.33	0.70	0.64	0.11
Mg	0.44	0.26	0.20	0.11	0.40	0.60	0.31	0.31	0.24
K	1.54	0.79	0.83	0.33	1.28	1.85	2.31	2.28	1.52
S	0.22	0.11	0.39	0.09	0.75	1.01	0.42	0.44	0.12

[1]DEMP (Alltech Inc., Nicholasville, KY).
[2]AMINOPLUS (Ag Processing, Inc., Omaha, NE).

Table 6. Analyzed AA composition of the alfalfa hay, corn silage, concentrate mixes, and YMP used in the lactation study.

AA, % of CP	Alfalfa Hay	Corn Silage	YMP[1]	Dried ground corn	Dried distillers grains with solubles	Corn gluten feed	Soybean meal, 46%	Expellers soybean meal[2]	Soyhulls
Arg	3.77	1.56	4.87	4.59	4.53	4.28	7.40	6.69	4.65
His	1.88	1.82	2.20	2.69	2.70	2.89	2.62	2.45	2.53
Ile	3.82	3.24	4.76	3.58	3.90	3.17	4.55	4.49	3.72
Leu	6.37	8.95	7.99	12.2	12.1	8.16	7.94	7.34	6.42
Lys	4.88	2.98	6.96	3.24	3.26	3.21	6.48	5.80	6.26
Met	1.27	1.56	1.64	2.13	2.07	1.35	1.39	1.38	1.27
Phe	4.38	3.50	4.36	4.81	5.00	3.49	5.09	4.86	3.72
Thr	3.82	3.11	4.57	3.36	3.83	3.33	3.86	3.63	3.30
Trp	1.00	0	1.17	0.78	0.83	0.71	1.44	1.45	0.76
Val	5.10	4.54	5.79	4.81	5.30	4.95	4.80	4.86	4.23

[1]DEMP, (Alltech Inc., Nicholasville, KY).
[2]AMINOPLUS (Ag Processing, Inc., Omaha, NE).

Table 7. Analyzed AA composition of the experimental diets used in the lactation study based upon individual ingredients[1].

	LF		HF	
AA, % of CP	NYMP	WYMP	NYMP	WYMP
Arg	3.06	3.04	3.14	3.12
His	1.83	1.84	1.95	1.96
Ile	2.95	2.98	3.37	3.40
Leu	7.45	7.50	8.23	8.28
Lys	3.15	3.19	3.64	3.69
Met	1.36	1.37	1.49	1.50
Phe	3.46	3.47	3.90	3.91
Thr	2.85	2.88	3.24	3.27
Trp	0.71	0.71	0.55	0.54
Val	3.98	4.02	4.53	4.57

[1]LF= low forage; HF = high forage; NYMP= no yeast-derived microbial protein; WYMP= with yeast-derived microbial protein.

Table 8. Penn State Particle Separator and Z Box results for experimental diets[1]

	LF		HF			Effect[2] (*P-value*)		
Item	NYMP	WYMP	NYMP	WYMP	SEM	F	YMP	F × YMP
Screen[3]	Percentage retained on each sieve							
Upper (19 mm)	4.62	4.00	6.89	6.55	0.82	<0.001	0.57	0.86
Middle (8 mm)	32.0	32.0	37.8	38.5	0.80	<0.001	0.63	0.64
Lower (1.8 mm)	32.2	33.1	31.5	31.7	0.80	<0.001	0.49	0.67
Bottom Pan	31.1	30.9	23.8	23.2	1.12	<0.001	0.68	0.87
pef[4]	0.44	0.44	0.54	0.51	0.022	0.002	0.64	0.45

[1]LF= low forage; HF = high forage; NYMP= no yeast-derived microbial protein; WYMP= with yeast-derived microbial protein.

[2]F=effect of forage concentration (LF vs. HF); YMP= effect of yeast-derived microbial protein (NYMP vs. WYMP); F × YMP= the interaction of forage and yeast-derived microbial protein.

[3]Particle size distribution of diets was measured using the Penn State Particle Separator (PSPS; Kononoff and Heinrichs, 2003).

[4]pef measured using the Z Box (W. H. Miner Agricultural Research Institute, Chazy, NY).

Table 9. Production measures for cows fed experimental diets[1].

	LF		HF			Effect[3] (*P-value*)		
Item	NYMP	WYMP	NYMP	WYMP	SEM	F	YMP	F × YMP
BW, kg	709.4	712.8	712.6	710.6	14.72	0.93	0.91	0.66
BW change, kg/d	0.68	0.48	0.36	0.48	0.176	0.36	0.84	0.35
BCS[5]	3.08	3.09	3.03	3.05	0.030	0.05	0.51	0.83
BCS change, per period	0.11	0.11	0.11	0.11	0.002	0.05	0.51	0.83
DMI, kg/d	26.98	26.84	24.68	25.80	0.928	0.004	0.35	0.24
Milk kg/d	39.89	40.37	38.39	37.27	1.087	0.005	0.68	0.31
Fat, %	3.84	3.68	3.98	3.89	0.155	0.04	0.14	0.70
Fat, kg/d	1.52	1.47	1.52	1.43	0.050	0.59	0.07	0.60
Protein, %	3.26	3.25	3.26	3.19	0.076	0.31	0.27	0.40
Protein, kg/d	1.30	1.31	1.25	1.18	0.034	0.001	0.24	0.16
Lactose, %	4.91	4.91	4.96	4.85	0.049	0.96	0.18	0.22
Lactose, kg/d	1.96	1.98	1.91	1.78	0.060	0.008	0.24	0.13
Total solids, %	12.92	12.75	13.11	12.74	0.251	0.52	0.05	0.44
Total solids, kg/d	5.15	5.12	5.03	4.73	0.128	0.01	0.12	0.18
MUN, mg/dL	10.12	10.19	11.43	10.78	0.304	<0.001	0.20	0.12
SCS[3]	4.39	4.69	4.63	4.92	0.374	0.32	0.22	0.99
ECM, kg/d[4]	42.10	41.67	41.22	39.23	0.977	0.04	0.13	0.33
ECM/DMI	1.50	1.47	1.54	1.44	0.046	0.82	0.02	0.11

[1]LF= low forage; HF = high forage; NYMP= no yeast-derived microbial protein; WYMP= with yeast-derived microbial protein.
[2]F=effect of forage concentration (LF vs. HF); YMP= effect of yeast-derived microbial protein (NYMP vs. WYMP); F × YMP= the interaction of forage and yeast-derived microbial protein.
[3]SCS=log(SCC).
[4]ECM= (0.327 × milk yield (kg)) + (12.95 × fat yield (kg)) + (7.2 × protein yield (kg)).
[5]Body condition score: 1=emaciated to 5 = obese (Wildman et al., 1982).

Table 10. Effect of experimental diets on ruminal pH, NH_3 concentration, and VFA concentration[1].

	LF		HF			Effect[2] (*P-value*)		
Rumen parameter	NYMP	WYMP	NYMP	WYMP	SEM	F	YMP	F × YMP
						----------*P-values*----------		
pH	6.05	5.95	5.97	6.01	0.132	0.74	0.46	0.09
NH_3-N, mg/dL	7.25	6.90	8.65	8.29	1.221	0.006	0.48	0.99
Acetate, mM	55.21	53.27	55.04	56.53	2.381	0.19	0.85	0.15
Propionate, mM	16.54	18.55	16.60	15.22	1.660	0.002	0.40	0.002
Isobutyrate, mM	0.65	0.65	0.71	0.73	0.040	<0.001	0.56	0.59
Butyrate, mM	8.02	8.31	8.58	8.95	0.526	0.008	0.14	0.86
Isovalerate, mM	1.21	1.23	1.41	1.46	0.216	<0.001	0.32	0.66
Valerate, mM	1.09	1.11	1.09	1.19	0.076	0.941	0.51	0.97
Total VFA, mM	82.73	83.13	83.44	84.21	4.657	0.63	0.75	0.92
Acetate, %	66.94	64.73	66.36	67.39	1.120	0.009	0.06	<0.001
Propionate, %	19.83	21.75	19.59	18.14	0.974	<0.001	0.40	<0.001
Isobutyrate, %	0.80	0.80	0.89	0.90	0.018	<0.001	0.59	0.77
Butyrate, %	9.68	9.94	10.19	10.54	0.161	<0.001	0.01	0.71
Isovalerate, %	1.45	1.48	1.69	1.73	0.168	<0.001	0.26	0.73
Valerate, %	1.30	1.30	1.29	1.30	0.041	0.87	0.87	0.86
Acetate:Propionate	3.47	3.08	3.43	3.75	0.203	<0.001	0.55	<0.001

[1]LF= low forage; HF = high forage; NYMP= no yeast-derived microbial protein; WYMP= with yeast-derived microbial protein.
[2]F=effect of forage concentration (LF vs. HF); YMP= effect of yeast-derived microbial protein (NYMP vs. WYMP); F × YMP= the interaction of forage and yeast-derived microbial protein.

Table 11. Effect of experimental diets on plasma metabolites[1].

	LF		HF			Effect[2] (*P-value*)		
Plasma metabolite	NYMP	WYMP	NYMP	WYMP	SEM	F	YMP	F × YMP
Glucose, mg/dL	67.01	62.20	64.83	63.53	2.421	0.86	0.21	0.47
PUN (arterial), mg/dL	12.81	13.01	15.28	14.71	0.597	<0.001	0.70	0.42
PUN (venous), mg/dL	11.56	11.84	14.05	13.79	0.482	<0.001	0.98	0.48

[1]LF= low forage; HF = high forage; NYMP= no yeast-derived microbial protein; WYMP= with yeast-derived microbial protein.
[2]F=effect of forage concentration (LF vs. HF); YMP= effect of yeast-derived microbial protein (NYMP vs. WYMP); F × YMP= the interaction of forage and yeast-derived microbial protein.

Table 12. Arterial plasma AA concentrations for cows fed the experimental diets[1].

	LF		HF			Effect[2] (*P-value*)		
AA	NYMP	WYMP	NYMP	WYMP	SEM	F	YMP	F × YMP
	----------µM/L----------							
EAA[3]								
Arg	73.16	66.62	67.78	64.04	3.040	0.14	0.06	0.60
His	45.53	44.79	45.78	40.64	2.287	0.20	0.06	0.15
Ile	126.38	113.53	110.62	103.56	4.401	0.005	0.03	0.51
Leu	154.98	145.06	138.71	130.27	5.347	0.005	0.08	0.89
Lys	83.08	73.33	73.52	66.60	3.209	0.007	0.006	0.62
Met	21.45	19.66	19.03	18.15	1.020	0.03	0.13	0.60
Phe	45.94	43.68	43.34	44.01	1.521	0.41	0.56	0.29
Thr	107.40	100.70	97.80	91.46	4.884	0.01	0.08	0.96
Trp	63.21	61.93	63.29	58.15	1.832	0.15	0.01	0.13
Val	255.45	237.30	229.54	213.86	9.167	0.002	0.03	0.87
NEAA[4]								
Ala	259.59	149.33	237.76	225.02	9.929	0.01	0.19	0.89
Asn	39.10	37.61	34.64	33.46	1.725	0.02	0.44	0.93
Asp	6.96	5.83	6.68	5.78	0.582	0.72	0.03	0.80
Gln	241.66	237.47	237.13	231.12	9.685	0.53	0.55	0.92
Glu	75.75	71.06	74.14	73.63	2.376	0.79	0.16	0.26
Gly	277.62	284.05	250.33	236.71	12.028	<0.001	0.73	0.33
Pro	87.43	86.60	82.73	78.42	3.448	0.05	0.43	0.59
Ser	92.63	91.51	85.94	83.52	3.528	0.04	0.60	0.85
Tau	50.15	52.68	50.98	45.19	2.846	0.14	0.47	0.07
Tyr	49.15	45.37	43.45	43.61	2.424	0.07	0.37	0.33
EAA	976.58	906.61	889.42	830.75	31.25	0.007	0.03	0.84
NEAA	1180.03	1160.50	1103.78	1056.45	35.62	0.01	0.33	0.67
BCAA[5]	536.81	495.89	478.87	447.70	18.17	0.003	0.03	0.77
TAA[6]	2156.61	2068.10	1993.19	1887.19	60.83	0.005	0.11	0.88

[1]LF= low forage; HF = high forage; NYMP= no yeast-derived microbial protein; WYMP= with yeast-derived microbial protein.

[2]F=effect of forage concentration (LF vs. HF); YMP= effect of yeast-derived microbial protein (NYMP vs. WYMP); F × YMP= the interaction of forage and yeast-derived microbial protein.

[3]EAA= Essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).

[4]NEAA= Nonessential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr).

[5]BCAA= Branched-chain AA (Val, Ile, and Leu).

[6]TAA= EAA + NEAA.

Table 13. Venous plasma AA concentrations for cows fed experimental diets[1].

	LF		HF			Effect[2] (*P-value*)		
AA	NYMP	WYMP	NYMP	WYMP	SEM	F	YMP	F × YMP
	----------µM/L----------							
EAA[3]								
Arg	35.52	29.23	34.33	31.61	2.177	0.68	0.004	0.23
His	27.36	26.43	28.33	24.45	1.905	0.65	0.04	0.20
Ile	74.99	64.19	64.90	61.91	3.067	0.03	0.02	0.17
Leu	78.91	71.47	70.76	67.69	4.059	0.11	0.16	0.55
Lys	28.12	22.37	25.00	22.14	2.045	0.32	0.01	0.39
Met	6.66	5.46	5.66	5.93	0.621	0.59	0.35	0.14
Phe	19.68	18.09	19.45	20.95	1.548	0.39	0.98	0.32
Thr	68.22	62.53	63.59	58.82	4.009	0.14	0.06	0.87
Trp	48.20	47.12	47.06	45.91	1.214	0.29	0.32	0.98
Val	179.18	162.45	161.93	149.56	6.987	0.01	0.02	0.71
NEAA[4]								
Ala	189.54	180.58	170.47	171.18	7.913	0.06	0.57	0.51
Asn	21.41	20.13	20.00	18.93	1.047	0.19	0.24	0.91
Asp	4.93	4.51	5.21	5.17	0.496	0.15	0.48	0.55
Gln	157.28	149.53	157.23	158.38	7.409	0.41	0.54	0.41
Glu	31.70	30.63	31.61	32.56	1.559	0.40	0.96	0.36
Gly	230.85	236.16	207.47	198.38	9.529	0.001	0.83	0.41
Pro	61.95	61.28	59.31	55.95	2.681	0.10	0.39	0.57
Ser	58.62	60.80	58.35	57.62	3.386	0.49	0.77	0.56
Tau	41.50	43.80	43.12	38.48	2.329	0.31	0.52	0.06
Tyr	25.51	22.19	22.82	23.47	2.093	0.65	0.39	0.21
EAA	566.84	509.34	521.00	488.97	21.522	0.08	0.02	0.49
NEAA	823.30	809.61	775.59	760.11	22.782	0.03	0.50	0.97
BCAA[5]	333.09	298.11	297.59	279.17	13.06	0.02	0.02	0.47
TAA[6]	1390.14	1318.95	1296.59	1249.08	36.28	0.03	0.10	0.74

[1]LF= low forage; HF = high forage; NYMP= no yeast-derived microbial protein; WYMP= with yeast-derived microbial protein.
[2]F=effect of forage concentration (LF vs. HF); YMP= effect of yeast-derived microbial protein (NYMP vs. WYMP); F × YMP= the interaction of forage and yeast-derived microbial protein.
[3]EAA= Essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).
[4]NEAA= Nonessential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr).
[5]BCAA= Branched-chain AA (Val, Ile, and Leu).
[6]TAA= EAA + NEAA.

Table 14. Arteriovenous differences of AA in dairy cows fed the experimental diets[1].

	LF		HF			Effect[2] (*P-value*)		
AA	NYMP	WYMP	NYMP	WYMP	SEM	F	YMP	F × YMP
	----------µM/L----------							
EAA[3]								
Arg	37.64	37.40	33.46	32.44	2.27	0.02	0.74	0.84
His	18.18	18.36	17.46	16.17	1.20	0.21	0.64	0.53
Ile	51.39	49.34	45.71	41.64	2.90	0.02	0.71	0.71
Leu	76.06	73.59	67.96	62.59	3.89	0.01	0.27	0.68
Lys	54.95	50.96	48.51	44.46	2.69	0.007	0.08	0.99
Met	14.79	14.20	13.37	12.22	0.75	0.01	0.18	0.65
Phe	26.26	25.59	23.90	23.06	1.25	0.04	0.51	0.94
Thr	39.18	38.17	34.21	32.64	2.55	0.03	0.58	0.90
Trp	15.01	14.81	16.23	12.24	1.72	0.58	0.09	0.13
Val	76.27	74.85	67.61	64.30	5.22	0.06	0.63	0.85
NEAA[4]								
Ala	70.05	68.74	67.29	53.84	6.60	0.12	0.19	0.28
Asn	17.69	17.48	14.63	14.53	1.24	0.01	0.89	0.96
Asp	2.03	1.32	1.47	0.61	0.48	0.19	0.10	0.87
Gln	84.38	87.95	79.89	72.74	6.43	0.11	0.77	0.38
Glu	44.05	4043	42.54	41.07	2.43	0.83	0.22	0.60
Gly	46.77	47.89	42.87	38.33	5.59	0.22	0.75	0.60
Pro	25.48	25.31	23.42	22.47	2.30	0.29	0.81	0.86
Ser	34.01	30.71	27.59	25.90	2.83	0.02	0.27	0.72
Tau	8.65	8.88	7.87	6.71	1.72	0.36	0.78	0.67
Tyr	23.64	23.18	20.62	20.14	1.43	0.02	0.72	0.99
EAA	409.73	397.27	368.42	341.78	22.59	0.02	0.34	0.73
NEAA	356.74	351.89	328.19	296.34	26.70	0.09	0.46	0.58
BCAA[5]	203.72	197.78	181.28	168.53	11.77	0.02	0.40	0.76
TAA[6]	766.47	749.15	696.61	638.12	48.31	0.05	0.39	0.64

[1]LF= low forage; HF = high forage; NYMP= no yeast-derived microbial protein; WYMP= with yeast-derived microbial protein.

[2]F=effect of forage concentration (LF vs. HF); YMP= effect of yeast-derived microbial protein (NYMP vs. WYMP); F × YMP= the interaction of forage and yeast-derived microbial protein.

[3]EAA= Essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).

[4]NEAA= Nonessential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr).

[5]BCAA= Branched-chain AA (Val, Ile, and Leu).

[6]TAA= EAA + NEAA.

Table 15. Extraction efficiency of AA in dairy cows fed the experimental diets.[2]

	LF		HF			Effect[3] (*P-value*)		
AA	NYMP	WYMP	NYMP	WYMP	SEM	F	YMP	F × YMP
	----------%----------					--------*P-values*-------		
EAA[4]								
Arg	51.61	56.15	50.22	50.40	2.23	0.03	0.14	0.17
His	40.94	43.07	38.58	40.96	2.67	0.29	0.29	0.95
Ile	40.27	43.09	41.66	40.29	1.83	0.66	0.66	0.20
Leu	49.25	50.61	49.63	48.19	20.6	0.58	0.98	0.44
Lys	66.53	69.95	67.03	66.37	2.12	0.34	0.39	0.21
Met	69.41	72.83	70.75	67.72	2.29	0.33	0.91	0.10
Phe	57.71	59.14	55.48	53.47	2.57	0.11	0.91	0.48
Thr	36.74	38.01	36.10	36.21	2.09	0.44	0.66	0.71
Trp	23.21	23.53	25.00	19.97	2.35	0.63	0.20	0.14
Val	29.69	31.19	29.65	29.68	1.65	0.63	0.63	0.65
NEAA[5]								
Ala	26.55	27.27	28.02	24.25	2.14	0.68	0.42	0.24
Asn	44.43	46.11	41.97	43.50	2.28	0.16	0.37	0.97
Asp	26.75	23.67	20.82	6.48	6.89	0.10	0.21	0.42
Gln	35.20	36.80	33.62	31.23	2.09	0.04	0.81	0.24
Glu	57.60	56.57	56.71	55.46	2.11	0.57	0.51	0.95
Gly	16.26	16.70	17.02	15.94	1.81	0.99	0.86	0.68
Pro	28.77	29.13	28.15	27.73	2.19	0.61	0.99	0.85
Ser	36.79	33.25	32.73	31.31	3.01	0.14	0.22	0.60
Tau	16.49	17.37	14.73	13.92	2.93	0.35	0.99	0.76
Tyr	48.66	51.87	48.43	46.45	2.94	0.22	0.79	0.26
EAA	41.76	43.62	41.62	40.82	1.74	0.33	0.73	0.38
NEAA	29.72	30.00	29.52	27.60	1.78	0.43	0.62	0.50
BCAA[6]	37.76	39.58	38.13	37.52	1.75	0.60	0.71	0.45
TAA[7]	35.12	35.89	34.80	33.35	1.68	0.35	0.82	0.46

[1]Extraction efficienty= AV difference/arterial concentration × 100.
[2]LF= low forage; HF = high forage; NYMP= no yeast-derived microbial protein; WYMP= with yeast-derived microbial protein.
[3]F=effect of forage concentration (LF vs. HF); YMP= effect of yeast-derived microbial protein (NYMP vs. WYMP); F × YMP= the interaction of forage and yeast-derived microbial protein.
[4]EAA= Essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).
[5]NEAA= Nonessential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr).
[6]BCAA= Branched-chain AA (Val, Ile, and Leu).
[7]TAA= EAA + NEAA.

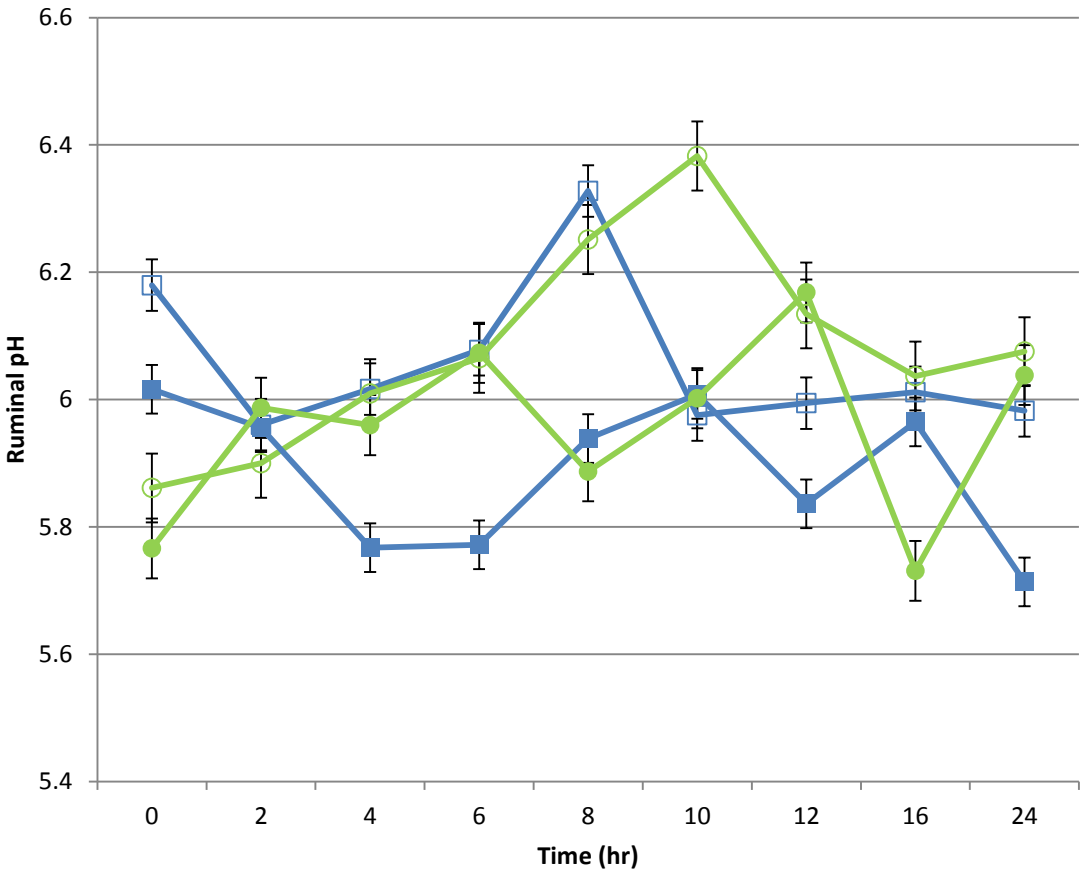

Figure 2. Ruminal pH of cows fed the experimental diets. Low forage no yeast-derived microbial protein (LFNYMP, □); low forage with yeast-derived microbial protein (LFWYMP, ■); high forage no yeast-derived microbial protein (HFNYMP, ○); and high forage with yeast-derived microbial protein (HFWYMP, ●). Effect of hour was significant (P<0.001). The interactions Forage (F) × Time, YMP × Time, and F × YMP × Time were not significant (P > 0.10).

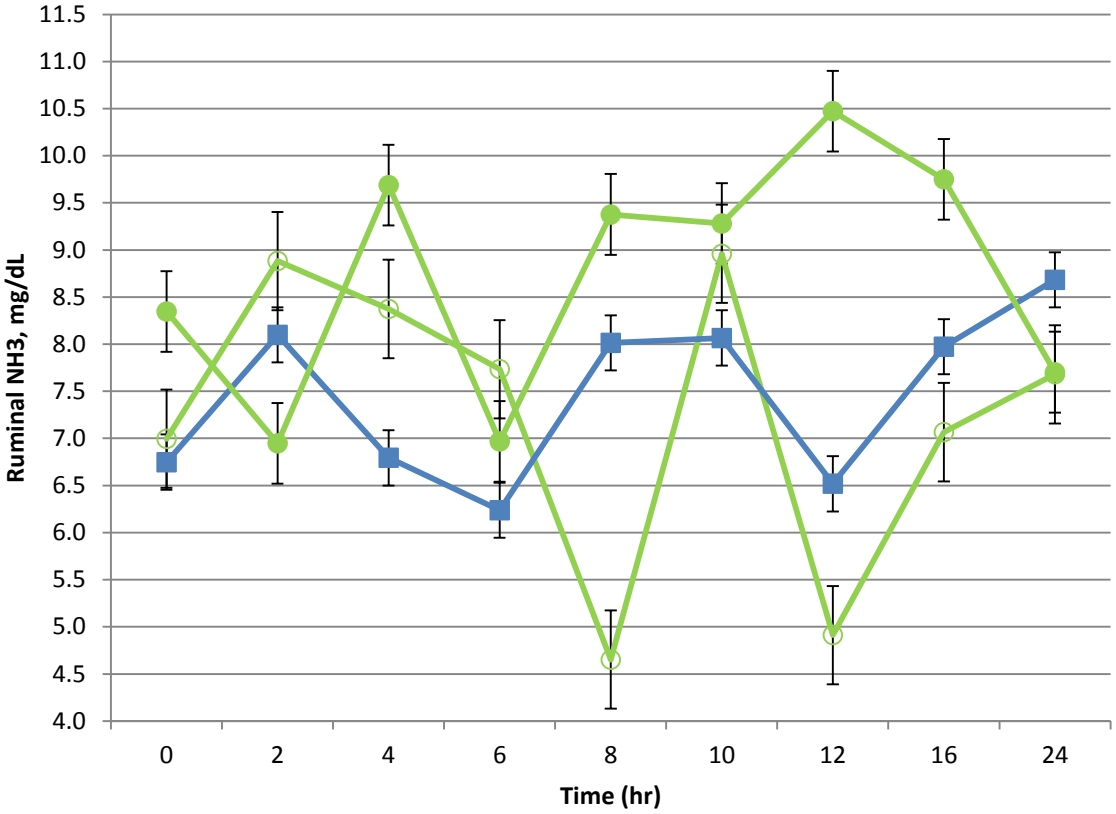

Figure 3. Ruminal ammonia of cows fed the experimental diets. Low forage no yeast-derived microbial protein (LFNYMP, □); low forage with yeast-derived microbial protein (LFWYMP, ■); high forage no yeast-derived microbial protein (HFNYMP, ○); and high forage with yeast-derived microbial protein (HFWYMP, ●). Effect of hour was significant (P<0.001). The interactions F × Time, YMP × Time, and F × YMP × Time were not significant (P > 0.10).

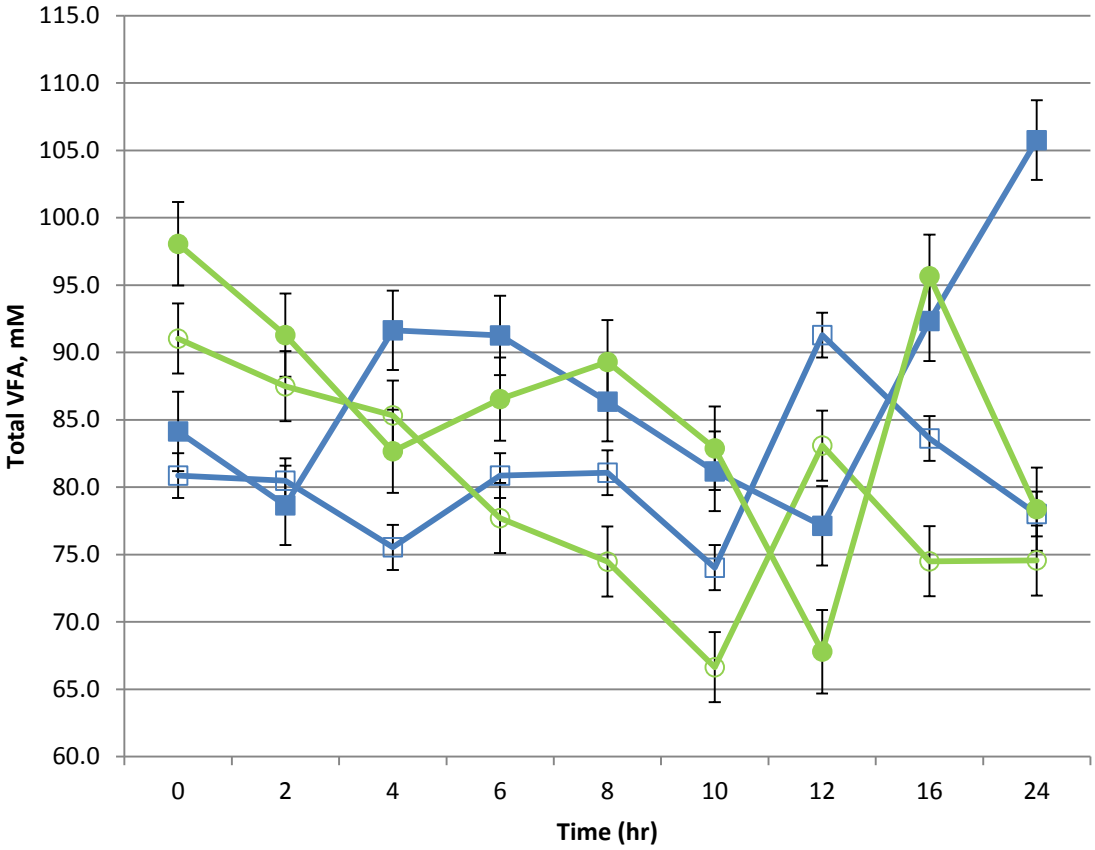

Figure 4. Total ruminal VFA concentration of cows fed the experimental diets. Low forage no yeast-derived microbial protein (LFNYMP, □); low forage with yeast-derived microbial protein (LFWYMP, ■); high forage no yeast-derived microbial protein (HFNYMP, ○); and high forage with yeast-derived microbial protein (HFWYMP, ●). Effect of hour was significant (P<0.001). The interactions F × Time, YMP × Time, and F × YMP × Time were not significant (P > 0.10).

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