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An evaluation of supplemental methionine sources for lactating dairy cows

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AN EVALUATION OF SUPPLEMENTAL METHIONINE SOURCES FOR LACTATING DAIRY COWS

BY

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To my wife, Melissa, for her support, encouragement, patience, and dedication in helping me pursue my Ph.D.

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ABSTRACT

AN EVALUATION OF SUPPLEMENTAL METHIONINE SOURCES FOR LACTATING DAIRY COWS

by

Ryan S. Ordway

University of New Hampshire, May, 2005

The most effective method for increasing supplies of MP-Met to dairy cows to increase milk protein production is to feed rumen-protected Met (**RP-Met**) products; however, not all RP-Met products are absorbed by the dairy cow in the same manner. Universally acceptable methods must be developed to determine the efficacy of different RP-Met products.

The first study was designed to compare the feeding of two sources of supplementary Met beginning 21 d prepartum and continuing for 140 d postpartum. The results indicated that feeding both sources of RPMet to periparturient dairy cows is effective in increasing milk protein production; however, clarification of their relative contributions to metabolizable Met is still needed.

The second study was designed to compare the effects of providing supplemental RP-Met in the form of Met analogs (MetaSmart) or DL-Met (Smartamine M) to lactating dairy cows fed Met-deficient or Met-adequate diets on plasma sulfur AA and milk protein concentrations. Results demonstrated that using changes in milk protein determine the amount of absorbable Met provided by MetaSmart relative to that of Smartamine M is not as precise as using changes in plasma sulfur AA concentrations.

This study also demonstrated that Met analogs are metabolized differently than DL-Met sources and that more research is needed to determine the best response criteria for determining the efficacy of Met analogs.

The third study was designed to determine the benefit of increasing MP-Met and MP-Lys concentrations on increasing milk and milk protein yields. Published studies in the *Journal of Dairy Science* were entered into the NRC (2001) model to generate predicted flows of MP, MP-Met, and MP-Lys (g/d). Plots were developed by regressing measured milk and milk protein yields on predicted supplies of MP, MP-Met, and MP-Lys (g/d). Results indicated that prediction equations based on NRC (2001) predicted flows of the first or co-limiting AA may be more accurate at predicting milk and milk protein yield than MP flows and predicting milk protein yield from flows of MP, Met and Lys is more accurate than predicting milk yield.

CHAPTER I

REVIEW OF LITERATURE

INTRODUCTION

Methionine (**Met**) and lysine (**Lys**) have been shown to be the two most often limiting amino acids (**AA**) for milk protein production in lactating dairy cows (Schwab et al., 1976; Rulquin et al., 1987; Schwab et al., 1992; NRC, 2001). The limitation in Lys and Met is the result of their low concentration in feed protein compared to the concentration found in milk protein and ruminally synthesized microbial protein (NRC, 2001).

Synthetic forms of supplemental Met, which have the capability of bypassing the rumen and being digested post-ruminally, have been developed. Dietary Lys concentrations can be increased through the addition of high Lys containing feeds such as blood meal, fish meal, soybean meal and protected soybean meal. However, these feeds, especially blood meal and fish meal which contain the highest concentrations of rumen bypass Lys, are limited in their usage resulting from problems related to availability, quality and consistency, cost, ethical and consumer perception, and palatability (Schwab et al., 1995). In the case of fish meal, an additional negative characteristic is a potentially adverse impact on flavoring characteristics of the milk if it is included in the diet in too high of a concentration. However, there are currently no synthetic forms of supplemental

rumen protected Lys available; therefore, nutritionists and dairy producers must rely on using blood meal, fish meal, or a soybean meal product to increase Lys concentrations to levels which are not limiting for milk protein synthesis.

IDEAL PROTEIN

In mammals, the plasma concentrations of AA are influenced by the nutritional or physiological conditions surrounding an animal (Bruhat et al., 1999). An alteration in the AA profile can occur if there is a deficiency of any one or more of the essential AA, a dietary AA imbalance, or an insufficient intake of essential AA (**EAA**) (Bruhat et al., 1999).

Ideal protein is a concept which has been utilized by the swine (NRC, 1998) and poultry (NRC, 1994) industries for many years, but has not been fully utilized and applied in the dairy industry. Ideal protein can be defined as the optimal pattern of dietary AA which corresponds to the needs of the animal (NRC, 1998). These needs, as defined by the Swine NRC (1998), are for maintenance, protein accretion, and milk protein synthesis.

Developing an ideal protein profile for swine is based on Lys which is used as a reference AA. The reasoning behind using Lys in swine nutrition is that it is predominantly used for protein synthesis and it is almost always the first-limiting AA. The Lys requirement has been the most extensively researched in swine. Lysine analysis in feeds is accurate and easy to conduct and no other limiting AA for growth is thought to be needed in greater concentration than Lys (Chung and Baker, 1992). Using Lys as the reference AA, ideal ratios of AA to Lys for maintenance, protein accretion, body tissue,

and milk protein synthesis have been developed (NRC, 1998). Lysine is given a value of 100 percent and the other EAA are given values higher or lower than Lys to indicate how much of a particular EAA is required. For example, milk protein synthesis has a Met requirement which is 26 percent of the Lys requirement.

If an ideal protein profile were to be developed for dairy cattle, Lys would also be the logical choice because, as in swine, Lys is one of the most limiting AA and no other limiting AA is thought to be needed in greater concentrations than Lys (NRC, 2001). This method of basing the requirement of EAA on the requirement for Lys has been adopted by the Dairy Cattle NRC (2001) but has only been extended to Met. The NRC (2001) suggests that the optimum Met requirement is approximately 33 percent of the Lys requirement or in other words, Met is required in approximately a 1:3 ratio with Lys.

Protein synthesis is an intricate process in which genetic information encoded in nucleic acids is translated into the 20-AA alphabet of polypeptides (McKee and McKee, 1999). Remarkably, this process occurs continuously with the same protein structure occurring each time. For example, the synthesis of milk protein results in the same AA sequence every time a protein is synthesized. As a result, the structure of milk protein cannot be altered by changing the AA profile of the diet because the same AA are required each time a protein molecule is synthesized. However, the extent to which protein is synthesized can be affected by the supply of the necessary AA required for synthesizing milk protein. The amount of AA available for milk protein synthesis is finite, therefore, an ideal balance or profile of EAA is necessary for maximizing milk protein synthesis. As soon as the availability of a specific EAA is limiting, milk protein synthesis is limited.

Without the necessary AA available, new protein cannot be synthesized and the excess AA which are not limiting in availability will be catabolized and the nitrogen (**N**) may be excreted into the environment. This excretion is not only energetically wasteful, but also environmentally damaging as N pollution is a growing concern in the United States (Powers, 2003).

When EAA are absorbed in the body in the profile which most closely matches the AA profile required by the animal for milk protein synthesis (i.e., the ideal profile of EAA), the efficiency of overall AA use is improved and the total amount of AA that needs to be absorbed can be minimized. As a result, the overall amount of rumen undegradable dietary crude protein (**RUP**) fed to lactating dairy cattle can be reduced. A reduction in RUP feeding will not only equate to a lower cost ration, but also to a less severe negative impact of milk production on the environment.

When the ideal profile of EAA is not provided to the animal, the efficiency of use of AA for milk protein synthesis is reduced resulting in lower milk protein production as well as wasting of N which ultimately increases the amount excreted into the environment. However, providing a less than ideal profile of EAA may also have another negative impact. In vitro research using human cell lines has demonstrated that when EAA are limiting, as occurs when an imbalanced profile of EAA is provided in the diet, changes in gene expression can occur. For example, depletion of arginine, cystine, and all EAA has been demonstrated in a dose-dependent manner to induce insulin-like growth factor-binding protein-1 mRNA and protein expression (Bruhat et al., 1999). Bruhat et al. (1999) has demonstrated that a leucine limitation induced CCAAT/enhancer-binding protein homologous protein (**CHOP**) mRNA and protein. The elevated mRNA levels

occur as a result of both an increased rate of CHOP transcription and an increase in mRNA stability. Overall, an AA limitation in conjunction with hormones, can play an important role in regulating gene expression. The extent to which this regulation affects normal metabolic functions is still unknown; however, the possibility exists that the effects may be profound.

For lactating dairy cow diets, it is imperative that the ideal concentrations of Lys and Met in metabolizable protein (**MP**) be determined so that diets can be formulated to meet these ideal concentrations to maximize protein synthesis through more efficient use of MP while minimizing the amount of protein in the diet. Noftsger and St-Pierre (2003) recently demonstrated that milk protein production can be maintained while reducing the amount of RUP fed. Their research indicated that feeding a more intestinally digestible form of dietary RUP, such as a high quality blood meal product, along with supplemental Met products and rumen-available Met (DL-2-hydroxy-4-methylthio butanoic acid (**HMB**)) maximized milk and milk protein yield as well as N efficiency in lactating cows. Of particular interest, was their finding that the low CP diet containing supplemental Met resulted in numerically greater milk yields and significantly greater milk protein concentrations compared to the high CP, high digestible RUP diet. The latter diet should have provided excess amounts of Lys and Met due to their greater supply in MP. These researchers concluded that lowering the amount of CP in the diet and properly balancing AA in the diet can reduce the amount of N excreted into the environment while maximizing production of milk and milk protein.

SUPPLEMENTAL METHIONINE AND LYSINE FEEDING

The seminal work of Schwab et al. (1976) indicated that Lys and Met are the first two limiting AA for milk protein synthesis in lactating dairy cows. In this study, researchers infused either single or mixtures of AA into the abomasum of lactating dairy cows and measured the response in milk yield, protein production, and fat production. Results indicated that Lys accounted for 16% of the total response in milk protein production and Lys plus Met accounted for 43% of the total response in milk protein production, but Lys plus Met accounted for only 7% of the total response in milk yield. The results indicated that Met was most likely co-limiting with Lys for milk protein synthesis in lactating dairy cows. Amino acid infusions did not have an effect on milk fat production, dry matter intake, or milk urea nitrogen. The effects on milk protein yield were accomplished via an increase in milk protein content, not through an increase in milk yield.

More than a decade later, Schwab et al. (1992) conducted another study which also indicated that Lys was the limiting or co-limiting AA along with Met for milk protein synthesis in lactating dairy cows. Similar to the study of Schwab et al. (1976), Schwab et al. (1992) infused either Met, Lys, Met+Lys, or casein into the duodenum of peak, early, mid, and late lactation dairy cows and observed the subsequent response in milk protein production. Their goal was to determine the sequence of Lys and Met limitation and to determine the effect of stage of lactation on the sequence of Lys and Met limitation. Their results indicated that during peak lactation, Lys was first-limiting and Met was second-limiting. During early lactation, Lys again appeared to be first-

limiting, however, Met did not appear to be limiting for protein synthesis. In midlactation, Lys was observed to be either slightly first-limiting or co-limiting with Met. During late lactation, there appeared to be no EAA deficiency, therefore, neither Lys nor Met were found to be limiting. Overall, results indicated that Lys and Met were the first and second limiting AA for milk protein synthesis throughout peak and mid-lactation, but only Lys was limiting during early lactation, and no AA were limiting during late lactation.

Despite the research which indicates that Lys is most likely the first-limiting AA for milk protein production or at least co-limiting with Met or another AA when low protein corn-based diets are fed, there are currently no rumen protected Lys (**RP-Lys**) products commercially available. This is due to the high cost associated with synthetically encapsulating Lys for commercial use. Therefore, to meet the Lys requirement for milk protein synthesis, feeds containing high concentrations of rumen undegradable Lys, such as blood meal and fish meal, are used. However, even though a product such as blood meal can provide adequate amounts of supplemental Lys to meet the requirement for maximizing protein production, it also supplies rather large amounts of the other EAA. As a result, an excess of EAA other than Lys and Met (blood meal is low in Met) are supplied which exacerbates the deficiency in Met. Fortunately, Met products are relatively inexpensive to produce and are commercially available. If a more economical commercially available form of RP-Lys does become available, the goal of feeding the ideal profile of EAA will become more attainable and the amount of excess N will be reduced. This is important not only for improving production efficiencies on farm, but also for public perception as products such as blood meal, which are derived

from animal proteins, are viewed negatively because of the possible perceived risk of bovine spongiform encephalopathy being spread to humans through cows which are fed animal proteins.

RUMEN-PROTECTED METHIONINE PRODUCTS

Several methods for ruminally protecting Met have been developed. These methods vary in their mode of action as well as their efficacy in providing supplemental post-ruminal Met. The methods which have gained commercial acceptance and regular use are Met products which contain a lipid-based or pH-sensitive coating resistant to ruminal degradation and Met hydroxy analogs which contain an hydroxyl group in place of the amino group of Met.

Improvements in N utilization with Met supplementation were observed 60 years ago by Loosli and Harris (1945). This is one of the earliest instances of the realization that AA, specifically Met, may be the key to improving growth and production efficiencies. Since this time, commercial development of RP-AA, specifically Met, have gained interest.

Feeding supplemental AA to ruminants has proven difficult because without protecting supplemental AA to allow them to escape ruminal degradation, free AA are quickly utilized by rumen microbes. Free AA in rumen fluid can arise from proteolysis of dietary protein, microbial synthesis, AA catabolism and microbial lysis. Despite the array of sources from which free AA in the rumen originate, studies by Velle et al. (1997) and Volden et al. (2001) have shown that the concentration of free AA in rumen fluid and

the contribution of free AA to intestinal digesta are negligible. This is the result of extensive deamination of any AA originating from hydrolysis of degradable protein in the rumen (Lewis and Emery, 1962). These results clearly demonstrate the need for developing Lys and Met products, as these two AA are commonly considered to be the first two limiting AA for milk and milk protein production (NRC, 2001).

Currently, only Met is available commercially as a ruminally-protected product. A combination of rumen-protected Lys and Met was available for a short period of time, but due to high costs associated with encapsulating Lys, it is no longer manufactured. The review papers of Schwab (1995; 2003) provide detailed information regarding the history, types, and efficacy of protected Met products. The following sections were adapted from Schwab (1995; 2003) and highlights details on the history, description, and efficacy of protected Met products.

Lipid-protected Methionine Products

Most attempts at encapsulating Met and Lys have focused on using a lipid-based coating containing carbohydrates and inorganic materials as stabilizers, as well as softening agents and fillers. These ingredients are readily available and are non-toxic, therefore, gaining government approval for use in dairy cow diets is generally not restrictive.

Lipid-protected Met products are effective at providing absorbable Met to the small intestine; however, the efficacy of these products depends on physical action and abrasion to degrade their outer coating. As a result, their protective coating does not completely prevent release of Met in the rumen. Therefore, depending upon conditions

in the rumen, more or less Met may be released resulting in the efficacy of the products being compromised. Additionally, storage of these products must be considered given their high content of fatty acids which are also susceptible to mechanical and thermal stresses (Schwab, 1995). Products with fatty acid-based coatings must find a balance between rumen protection and intestinal release so that a maximum amount of Met is released in the small intestine (Schwab, 1995; Schwab, 2003).

The original Met product was produced in the 1960's by Delmar chemicals of Canada. Their product consisted of a core of DL-Met with a coating of colloidal kaolin and tristearin wrapped around it in a continuous film of tristearin with the final product containing 20% Met, 20% colloidal kaolin, and 60% tristearin. The product was a small bead with a diameter of 0.3 to 1.0 mm and a specific gravity of 1.18-1.20g cm^{-3}. Research demonstrated that approximately 60-65% of the Met bypassed the rumen and was available for intestinal absorption, resulting in only 12-13% of the original as fed product being bioavailable Met.

Several years later, another product called Ketionin was introduced by Rumen Kjemi A/S of Oslo, Norway. Ketionin contained a greater amount of Met and had an improved protective coating which allowed for a greater amount of Met to be released and absorbed in the small intestine compared to the Delmar product. Ketionin was available in 2.1 and 3.5 mm particle sizes and contained 30% DL-Met, 2% glucose, 4% stabilizer, antioxidant and flavoring agents, 6% $CaCO_3$, and 58% tristearin and oleic acid. Research indicated that approximately 80% of Ketionin escaped ruminal degradation and 20% was lost in the feces of lactating cows. Other research conducted with non-lactating, growing heifers indicated that approximately 72% of the product escaped ruminal

degradation and 19% was lost in the feces. Overall, approximately 19% of the original as fed product was bioavailable Met when fed to lactating dairy cows.

Mepron M85 (Degussa Corporation, Germany) consists of a core containing a minimum of 85% DL-Met coated with thin layers of ethylcellulose combined with stearic acid. The pellets are small and cylindrical (1.8 mm diameter, 3-4 mm length), have a density of 1.2g cm^{-3}, and have the thinnest coating on the corners. The final product contains 85% DL-Met, 8.5% nonstructural carbohydrates, 3.5% neutral detergent fiber, 1.5% ash, 1.0% moisture, and 0.5% crude fat. This product contains the highest concentration of Met available in a commercially produced Met product. Its' mode of degradation relies on physical action and abrasion to wear away the ethylcellulose outer coat as a result of ethylcellulose being resistant to enzymatic digestion. The result is a slow-release Met product which begins degradation in the rumen and then slowly releases Met in the small intestine. The Met bioavailability value of Mepron M85 is approximately 40%.

Met-Plus (Nisso America, Inc.) consists of a core containing a minimum of 65% DL-Met which is embedded in a matrix of calcium salts of long-chain fatty acids, lauric acid, and butylated hydroxytoulene which is used as a fatty acid preservative. This product also relies on physical action and abrasion in the rumen to wear away the outer coating so the Met can be slowly released in the small intestine. Met-Plus has been the least researched of the Met products and as a result, only a few studies have been conducted in which a Met bioavailability value was determined. Bach and Stern (2000) determined a bioavailability value of approximately 40%, while Olley et al. (2004)

determined a bioavailability value of approximately 33%, resulting in an average bioavailability value of approximately 37%.

pH-sensitive-protected Methionine Products

The most technologically advanced surface-coating product is the rumen-inert pH-sensitive synthetic polymer coating. Not only is this coating technology the most advanced, but it has been shown to be the most effective in terms of rumen-protection and release of Met in the small intestine. These polymers are unaffected by the rather neutral pH of the rumen and by enzymatic digestion. Instead, they are sensitive to the low pH environment of the abomasum and small intestine. The coating resists degradation in the rumen, but is quickly degraded in the abomasum as a result of the large decline in pH as the product flows from the rumen to the abomasum. This type of product allows for rapid release and delivery of Met in the small intestine.

The Eastman Kodak Company (USA) pioneered the development and use of commercially produced pH-sensitive polymer coatings. Originally, two types of polymers were tested, DL-Met coated with cellulose propionate morpholinobutyrate and DL-Met or L-Lys coated with a copolymer of styrene and 2-methyl-5-vinyl pyridine. Both showed promising results with research indicating that under in vitro conditions where pH was maintained near 5.5 for 17 h and in nylon bags incubated in the rumen of sheep for 24 h, the DL-Met coated with cellulose propionate morpholinobutyrate product was greater than 96% resistant to ruminal degradation as indicated by the amount of product remaining after incubation. Similarly, the DL-Met or L-Lys coated with a copolymer of styrene and 2-methyl-5-vinyl pyridine product remained 94-95% resistant

to degradation when incubated for 24 h in an acetate buffer of pH 5.4. Under more severe acidic conditions, such as those found in the abomasum, the DL-Met coated with cellulose propionate morpholinobutyrate product was completely degraded within 30 min, while the DL-Met or L-Lys coated with a copolymer of styrene and 2-methyl-5-vinyl pyridine product was approximately 95% degraded after 1 h in pH of 2.9.

The major concern regarding the coating technologies was that the coating of these products would degrade when mixed with acidic feeds such as silages. Using the copolymer of styrene and 2-methyl-5-vinyl pyridine, research by Papas et al. (1984) reported Met recovery values of 80% and 70% after incubation with a silage diet of pH 4.6 for 2 and 18 h, respectively. Other researchers (Polan et al., 1991) using the same copolymer consistently observed that Met and Lys incubated in a silage based diet for 8-12 h greatly resisted degradation and recorded recovery values exceeding 90%.

Currently, Adisseo Animal Nutrition (Antony, France) holds the patent rights to the polymer technology and is currently manufacturing a commercially protected form of Met called Smartamine M. Previously, they also marketed a protected Lys and Met product called Smartamine ML, but have discontinued its' production.

Smartamine M is a surface coated Met product containing a minimum of 75% DL-Met. The pellets are 2-mm in length and are slightly rounded with no hard edges. They consist of a core of 75% DL-Met and ethylcellulose which is coated with stearic acid containing droplets of 2-vinyl-pyridine-co-styrene. This copolymer contributes only 3% of the final weight of the product, but appears to alter the stereochemistry of the stearic acid in such a way that the outer surface's resistance to ruminal degradation is improved. However, under very acidic conditions, such as those found in the abomasum,

the copolymer is rapidly solubilized and degraded, thus allowing for rapid release of the Met in the abomasum and subsequent availability for absorption in the small intestine. Smartamine M is considered to be the most efficacious Met product and has a Met bioavailability of 80% (Robert et al., 2002; Rulquin and Kowalczyk, 2003).

Methionine Analogs

Another method of supplementing Met is to use Met analogs. Methionine analogs are similar to Met except that they have a non-nitrogenous group substituted for the α-amino group. The Met analog which has been studied most extensively is the acid form of the analog or more specifically, 2-hydroxy-4-methylthio butanoic acid (**HMB**). More recently, a derivative of HMB, 2-hydroxy-4-methylthio butanoic acid isopropyl ester (**HMBi**) was developed which has been shown to provide a greater amount of metabolizable Met than HMB (Schwab et al., 2005a,b).

In the late 1960's, Griel et al. (1968) observed a stimulatory effect on milk production when HMB was fed to lactating dairy cows. This finding was coincidental as the original objective of the study was to feed HMB as a preventative supplement for ketosis. However, these researchers observed an increase in milk production with HMB feeding. These researchers hypothesized that either HMB had a stimulatory effect on rumen microorganisms or that part of the HMB was absorbed across the gut and converted to Met based on observed increases of free Met concentration in plasma and may have beneficial effects if supplemented in the diet.

The effects of HMB on ruminal fermentation have been varied. For example, Deswysen et al. (1991) fed 0 or 5 g/d HMB to young and mature wethers. Significant

increases in apparent DM, OM, and CP digestibility in young wethers but observed no effect on these parameters in mature wethers was observed. Using continuous culture fermenters, Vázquez-Añón et al. (1999) reported that HMB did not elicit any changes in rumen fermentation. In a more recent study, Vázquez-Añón et al. (2001) increased the concentration of HMB from 0 to 0.88% of DM and again did not observe any changes in DM, CP, ADF, or starch and sugar digestibility or VFA concentration. However, they did observe increases in NDF digestibility, ruminal ammonia-N concentration and bacterial protein synthesis with increasing concentration of HMB.

One of the most controversial effects of HMB is its' ability to stimulate milk fat synthesis. Results of several studies have reported increased milk fat synthesis with HMB supplementation (Patton et al., 1970b; Polan et al., 1970; Lundquist et al., 1983), however, several other studies have indicated no impact of feeding HMB on milk fat synthesis (Pullen et al., 1989; Phillips et al., 2003; Piepenbrink et al., 2004; Noftsger et al., 2005; Schwab et al., 2005a,b). The studies conducted by Noftsger et al. (2005) and Schwab et al. (2005a,b) utilized Latin square designs while the other studies utilized randomized block designs, which does not indicate that the effects of HMB were impacted by the statistical design of the studies. Early hypotheses of the mechanism by which HMB may affect fatty acid synthesis focused on an increase in fatty acid synthesis in the rumen and/or an alteration in the fatty acid composition of plasma lipoproteins (Griel et al., 1968; Patton et al., 1970a,b; Polan et al., 1970). More recent research has indicated that HMB does not effect lipid metabolism (Pullen et al., 1989; Phillips et al., 2003; Piepenbrink et al., 2004); as there was no effect of HMB supplementation on nonesterified fatty acids (**NEFA**), glucose or triglyceride (**TG**) concentrations. The only

significant effect of HMB was an increase in the incorporation of labeled ^{14}C (from labeled palmitic acid) in milk fat (Pullen et al., 1989). However, no effect of MHA on NEFA metabolism or on NEFA and TG plasma concentrations was observed. Subsequently, no differences in milk or milk fat production were observed. The authors did not speculate as to whether there was any significance of the increased incorporation of labeled ^{14}C in milk fat; however, Piepenbrink et al. (2004) suggested that this effect may have resulted in a greater use of NEFA for milk fat synthesis occurred (Piepenbrink et al., 2004). In the study by Pullen et al. (1989), the authors acknowledged that the concentration of TG in plasma was only approximately 10% of what they had expected. They attributed to the apparent loss of TG concentration to an incomplete recovery in the HPLC fractions. They stated that the quantity and radioactivity of TG were determined in the same fraction which means the error in recovery did not affect TG specific activity. However, they did point out that any changes in TG concentration which may have occurred due to treatment or lactation effects would influence the estimates of the percentage of NEFA incorporated into TG since the authors assumed a constant plasma TG concentration. This error is most likely the reason for the observed effect of HMB on the incorporation of palmitate into milk fat.

Altering the VFA profile in the rumen may be another method through which HMB can increase milk fat synthesis. Robert et al. (2003) recently demonstrated that the amount of HMB available in HMBi for ruminal degradation is sufficient to cause an alteration in VFA pattern of non-lactating dairy cows. Noftsger et al. (2003) observed an increase in the acetate:propionate ratio when incremental amounts of HMB was added to diets fed to continuous culture fermenters. This increase was the result of a linear

decrease in propionate concentrations as no effect on acetate concentrations was observed. Furthermore, no effect of incremental amounts of HMB was observed on fiber digestibility, NH_3 concentrations, or flows of nitrogen from the rumen fermenter. Noftsger et al. (2005) did not observe any changes in VFA concentrations in the rumen of lactating cows nor did they observe any changes in rumen pH, NH_3, total VFA concentrations, or flow of NH_3-N, microbial N, or nonbacterial non-ammonia N to the omasum with HMB, HMBi, or DL-Met supplementation. They did, however, observe an increase in apparent organic matter digestibility as well as an increase in NDF digestibility with HMB, HMBi, or DL-Met supplementation. Addition of HMBi resulted in greater concentrations of milk protein, but no effect of feeding HMBi, HMB or DL-Met were observed for milk yield, milk fat concentration or milk fat yield. Given the more recent research results indicating that HMB does not affect lipid metabolism, as well as the number of studies which have not reported any effects of HMB on milk fat synthesis, it would appear that HMB does not provide a significant benefit to increasing milk fat synthesis or improving fatty acid metabolism. Despite the results which demonstrate that HMB can alter VFA patterns in the rumen, this alteration does not appear to affect fatty acid metabolism or milk fat synthesis in vivo (Pullen et al., 1989; Phillips et al., 2003; Piepenbrink et al., 2004; Noftsger et al., 2005; Schwab et al., 2005a,b).

The most likely explanation for this is that when HMB is fed in a total mixed ration (**TMR**), the majority of the product is degraded rapidly in the rumen and does not pass into the abomasum or get absorbed across the rumen wall. When HMB is pulse-dosed into the rumen, the enzymes in the rumen which degrade HMB become saturated,

which results in an abnormally large amount escaping the rumen compared to when it is fed in a TMR and more physiological conditions exist in the rumen.

Researchers (Belasco, 1980; Patterson and Kung 1988) have demonstrated that HMB is less sensitive to ruminal degradation than unprotected DL-Met, however, the extent to which HMB is absorbed and converted to metabolizable Met and is used for milk protein synthesis, remains uncertain. There have been large variations in the bioavailability of HMB as reported in the scientific literature. Several researchers have not observed an increase in milk protein synthesis in cows fed Met-deficient diets supplemented with HMB (Piepenbrink et al., 2004; Schwab et al., 2005a,b; Noftsger et al., 2005). These results indicate that HMB is most likely degraded in the rumen with very little being absorbed across the rumen wall and subsequently converted to Met for milk protein synthesis. In a study by Jones et al. (1988), researchers concluded that 99% of HMB was either degraded or altered in the rumen as a result of observing less than 1% HMB recovery in duodenal digesta samples. However, other researchers have observed 22 to 50% of dietary HMB escaping ruminal degradation (Koenig et al. 1999, Vásquez-Añón et al., 2001; Koenig et al., 2002). These differences are most likely attributable to the method of HMB administration or supplementation to the cow (i.e., administered via the diet or pulse-dosed in the rumen) as it appears that residence time has a large effect on the degradability of HMB in the rumen (Vásquez-Añón et al., 2001). As previously discussed, the enzyme system in the rumen for degrading HMB are most likely saturated, which greatly increases the rumen-bypass characteristics of the product compared to normal physiological doses.

Robert et al. (2001d) examined the bioavailability of eleven different esters of Met and HMB using a blood kinetics method to determine which, if any, of the esters was most effective at increasing plasma Met concentrations. They concluded that HMBi resulted in the greatest bioavailability of the esters examined. In addition to this study, subsequent research has demonstrated that HMBi is effective at increasing plasma Met concentrations (Robert et al., 2001c; Ordway et al., 2004; Schwab et al., 2005b) and milk protein concentrations (Guyot et al., 2004; Ordway et al., 2004; Noftsger et al., 2005; Schwab et al., 2005a). Both Schwab et al. (2005a,b) and Noftsger et al. (2005) observed that HMBi is not only an effective source of bioavailable Met, but that it is more effective at increasing plasma Met and milk protein production than HMB.

Alimet (Novus International, Inc., St. Louis, MO, USA) and Rhodimet AT88 (Adisseo, Antony, France) are both liquid forms of HMB which are available commercially and are used extensively as Met supplements in the poultry and swine industries. Both products are also labeled for use in dairy cow rations, however, the extent of their use in dairy rations is considerably less than in poultry and swine rations. Alimet is promoted as an Met source, but as previously stated, its' ability to resist ruminal degradation is questionable. The bioavailability value of HMB is considered to be negligible (Schwab et al., 2005a).

Adisseo (Antony, France) has recently introduced a commercially available form of HMBi under the trade name MetaSmart. As previously stated, it has been shown to be a more efficacious source of bioavailable Met and is being marketed as such. It is currently available for use in dairy cow rations and is considered to have a bioavailability of approximately 50%.

METHIONINE ANALOG VS. DL-METHIONINE METABOLISM

Research has demonstrated that not only is supplemental Met utilized differently than Met analogs within species, but it is also utilized differently between ruminants and non-ruminants as well (Baker, 1986; Lobley et al., 2001). Traditionally in non-ruminants, it has been postulated that HMB is absorbed from the gastrointestinal tract and transported to the liver where it is removed from circulation and transaminated to form L-Met (Bottje et al., 1998, Robert et al., 2001b). However, more recent research (Wester et al., 2000a,b) has demonstrated, using sheep, that this pathway may be incorrect, especially in ruminants.

Wester et al. (2000a) infused labeled (1-13C)Met into the jugular vein of growing lambs for 12 h and then from 3 h onward, infused successive 3 h infusions of 0.55 mg/min saline and 4.4 mg/min HMB into the mesenteric vein. They took continuous plasma samples every 20 min during the last 80 min of each infusion. They recovered all of the infused HMB at the portal vein, but 25% was subsequently extracted by the liver. Results indicated that portal appearance of Met and Cys was unaltered by HMB infusion, however, net splanchnic output of Met decreased while Cys output increased with increasing rates of HMB infusion. Although dietary Met did not appear to be released into peripheral circulation, arterial concentrations of Met as well as the Met irreversible loss rate linearly increased. They calculated that the increase in Met irreversible loss rate was equivalent to 40% of the HMB delivered past the liver which was metabolized by peripheral tissues and entered the plasma Met pool. These researchers concluded that the liver does not secrete the Met which was transaminated from the extracted HMB,

however, the liver may increase Cys output. It appears from their results that HMB is metabolized extensively by peripheral non-hepatic tissues.

In another study conducted by Wester et al. (2000b), unlabeled HMB was infused into the abomasum of growing lambs for 24 h followed by a 6 h infusion of labeled (1-13C)HMB into the abomasum with another infusion of ($2H^3$)Met into the mesenteric vein. Continuous samples of plasma were collected at 30-min intervals during the last 2 h of infusion from the aorta, portal and hepatic veins. Recovery of infused HMB was 75% at the portal vein with 36% being subsequently extracted by the liver. They observed that HMB contributed 10% to overall irreversible Met loss rate which was equivalent to 40% of absorbed Met and similar to the concentration of HMB observed post-hepatically. Results indicated that labeled (1-13C)Met enriched arterial plasma more than portal or hepatic plasma and the ratio of $13C:2H^3$ Met enrichments were also greater in arterial compared to portal or hepatic plasma. The authors concluded, based on analysis of $13C:2H^3$ free Met enrichments from visceral tissues where Met synthesized from HMB represented 22 to 26% of Met present these tissues, that post-splanchnic tissues were involved in the synthesis of Met from HMB and the involvement was in the order of kidney > liver > rumen > jejunum > duodenum > ileum. They also found that the lungs, brain, muscle and skin also synthesized Met from HMB but this accounted for less than 5% of intracellular Met. This study provides further evidence that HMB is being utilized by peripheral tissues to meet their Met requirements. It also provides evidence that any increases in plasma Met concentrations observed with supplemental MetaSmart feeding (HMB is not assumed to bypass the rumen under normal feeding conditions) is most likely the result of a Met-sparing effect and not necessarily the result

of a net export of Met from tissues such as the liver which transaminates HMB into L-Met.

METHIONINE, CYSTEINE, AND HOMOCYSTEINE METABOLISM

The metabolism of Met, cysteine (**Cys**), and homocysteine (**Hcys**) is a complex interconnected network of metabolic pathways. The interactions of these three AA is complicated even further when Met analogs such as HMB or MetaSmart are fed. The overall pathways of Met and Hcys metabolism are depicted in Figure 1. From this figure, it is apparent that there are several other cofactors which are intimately related to these pathways, such as vitamin B_{12}, folic acid (a B-vitamin), serine (**Ser**), and ATP. Choline, a methyl group donor, is another potentially vital cofactor for the synthesis of Met from S-adenosylmethionine (**SAM**) if Met becomes severely deficient in the diet.

As shown in Figure 1, there are methyl acceptors and resultant methylated products in the conversion of SAM into S-adenosylhomocysteine. Examples of methyl acceptors and their subsequent methylated products are: phosphatidylethanoloamine and phosphatidylcholine, norepinephrine and epinephrine, guanidinoacetate and creatine, and γ-aminobutyric acid and carnitine.

Homocysteine and Ser condense to form Cys in the transulfuration pathway (Figure 1). The carbon skeleton of Cys is derived from Ser while the sulfhydryl group is transferred from Met via Hcys. This process intimately relates the transulfuration and transmethylation pathways. Homocysteine can also be synthesized from homoserine and Cys which can then be synthesized into Met in the transmethylation pathway (Figure 1).

METHODS OF DETERMINING METHIONINE BIOAVAILABILITY

Perhaps, the most important aspect of designing Met analogs and Met products is to be able to determine their bioavailability. Once a bioavailability value has been generated, it is possible to compare the efficacy of one product against another and to determine if the product is providing the animal with post-ruminal absorbable Met.

Several methods have been used to determine the effectiveness of Met analogs and Met products in providing absorbable Met to lactating dairy cows. These include in situ incubation coupled with an enzymatic in-vitro procedure, spot-dosing the Met product and determining the changes in plasma Met using a technique called area-under-the-curve (**AUC**). Other methods include feeding the Met product to cows fed Met-adequate diets and observing the subsequent changes in plasma Met and feeding the Met product to cows fed Met-deficient diets and observing the subsequent changes in milk protein production. Regardless of the methodology employed, it is essential that the procedure is repeatable and that it provides a true measure of differences in Met availability to the animal. Each technique has its' benefits and drawbacks. To date, there is no single procedure for determining the bioavailability of Met products which is universally accepted. However, some techniques are considered more effective than others over a wide-range of Met products.

There are two major categories for determining the bioavailability of Met products. The first category involves using changes in plasma Met concentrations while the second category utilizes changes in milk protein concentration.

Within the category of using changes in plasma Met concentrations to determine the bioavailability of Met products, two techniques have been developed. The first technique is called AUC (Robert et al., 2001a,d; Robert et al., 2002; Graulet et al., 2005). This technique relies on determining a basal plasma Met concentration for each cow on the study by generating a mean plasma Met concentration from plasma Met samples taken over a period of several hours prior to pulse-dosing Met with the Met product being tested. The basal plasma Met concentrations are then subtracted from the plasma Met concentrations quantified for each cow after Met supplementation. The resultant values correspond to increases in plasma Met concentrations resulting from Met supplementation. A curve based on increases in plasma Met concentrations over time is determined using these values. The AUC is calculated mathematically by multiplying the average increase in plasma Met concentration between two consecutive sampling times and the length of time between the samplings. Total AUC is determined as the sum of the individual AUC calculated along the sampling kinetics. In order to calculate a bioavailability value for the Met product being tested, a Met digestibility value must be calculated using the resultant AUC values. Robert et al. (2001a) derived an equation to model this. Their original equation was in the form of [Digestible Met = a x ln (1 + AUC / b)]. In a more recent study, Graulet et al. (2005) observed large variations in basal plasma Met concentrations among individual cows. As a result, a correction factor was added to the equation to help account for differences in the basal plasma Met concentrations (**BPMC**) prior to infusion or feeding of the supplemental Met source. The first parameter, a, which was a constant value of (19.7406) in the initial equation was replaced by a variable coefficient, A, which is calculated from each individual BPMC

value, where A = -0.5895 x BPMC + 32.5073. Therefore, the final adjusted equation is [Digestible Met = (-0.5895 x BPMC + 32.5073) x ln (1 + AUC / 925.74). This equation allows for a corrected AUC value by accounting for variation in the basal plasma Met concentration of individual cows prior to infusion or supplementation of Met products. However, it is important to note that this equation was based on data derived from non-lactating dairy cows. This equation has not been evaluated using lactating dairy cows; therefore, the adjustment factor which accounts for variation in basal plasma Met concentration may be different depending upon state of lactation.

The second technique utilizes changes in plasma Met concentrations when Met products are fed in incremental amounts to cows receiving a Met-adequate diet (Olley et al., 2004; Schwab et al, 2005b). Lactating cows are fed a diet adequate in Met to ensure that absorption of additional supplies or sources of Met will elicit increases in plasma Met concentrations and that the increases in plasma will not be compromised by increased uptake of Met by body tissues, such as the mammary gland. Methionine bioavailabilities are calculated by regressing plasma Met concentrations on levels of supplemental Met. Smartamine M has been used as the reference Met source with an assumed bioavailability value of 80% (Robert et al., 2002; Rulquin and Kowalczyk, 2003). The slopes of the other Met sources being tested are divided by the slope for Smartamine M to obtain a bioavailability relative to that of Smartamine M.

The second category of determining the bioavailability of Met products utilizes changes in milk protein production when cows are fed a diet deficient in Met and supplemented with increasing levels of Met (Schwab et al., 2005a). This method requires that cows are fed a Met deficient diet to ensure that a response in milk protein to Met

supplementation will be observed. Changes in milk protein concentrations are regressed on the increasing levels of supplemental Met. Similar to the method of Schwab et al. (2005b), Smartamine M was used as the reference Met source with an assumed bioavailability of 80% (Robert et al., 2002; Rulquin and Kowalczyk, 2003). The slopes of the other Met sources being tested are divided by the slope for Smartamine M to obtain a bioavailability value relative to that of Smartamine M.

A combination approach of using in situ incubation and the in vitro three-step procedure of Calsamiglia and Stern (1995) has been examined by Bach and Stern (2000) to determine the ruminal degradation and intestinal digestion of Met products. The limitation to using this combination approach is that it cannot be used for soluble forms of Met sources, such as HMB and HMBi, because the pore size of the in situ bags allows the product to escape (Bach and Stern, 2000). Overall there appears to be a strong correlation between in situ and in vitro estimates of bioavailability and bioavailability estimates obtained from measuring changes in plasma Met concentrations and measuring their area under the curve. Both techniques have utility and some variation in results should be expected given that both bioavailability values are estimated from observed changes in plasma Met concentrations.

CHALLENGES AND LIMITATIONS RELATED TO USING CURRENT BIOAVAILABILITY METHODOLOGIES

There are many challenges and limitations involved with determining the bioavailability of Met analogs and Met products. Using changes in milk protein

concentration is an indirect approach and is dependent upon the Met status of the basal diet. If the diet is not deficient in Met, supplying additional amounts or sources of Met would not be expected to increase milk protein concentrations. This makes it impossible to calculate bioavailability values based on linear increases in slope of milk protein concentrations in response to feeding incremental amounts of Met analogs or Met products. This problem was encountered in a recent study by Schwab et al. (2005a). No linear increases in milk protein concentrations in response to feeding incremental amounts of Smartamine M, HMB, HMBi, or Mepron M85 were observed. Therefore, bioavailability calculations based on changes in slope of milk protein concentrations could not be made.

Another disadvantage of this methodology is the large variation of the bioavailability calculation. Schwab et al. (2005b) calculated a Met bioavailability for HMBi based on changes in milk protein concentrations of approximately 42 ± 16.0% and a bioavailability for MetaSmart relative to that of Smartamine M of 53 ± 20.0%, assuming an 80% bioavailability for Smartamine M. This variation (based on calculated 95% confidence intervals) could most likely be reduced through more replication or more blood sampling time points which would help reduce the fluctuations in plasma Met concentrations which occur throughout the day (Graulet et. al., 2005). Despite these disadvantages, a decided advantage of using changes in milk protein concentration is its' applicability. While it may not be the most precise method of determining Met bioavailabilities in terms of standard error, it does provide a practical value for dairy nutritionists and producers who may be looking for responses in actual production parameters as a means by which to gauge the efficacy of Met analogs or Met products.

The use of changes in plasma Met concentrations in response to feeding incremental amounts of Met analogs or Met products also has challenges and limitations. Similar to using changes in milk protein concentrations, using changes in plasma Met concentrations may be dependent upon the Met status of the basal diet. Theoretically, the more Met-adequate the basal diet is; the more pronounced the response in plasma Met will be. Assuming the Met requirement has been met; tissues would not be expected to continue to utilize Met from the plasma, thus allowing Met to accumulate in plasma. If the basal diet is not as adequate as was predicted, there may not be a significant linear increase in plasma Met concentrations resulting in the inability to calculate bioavailability values. Schwab et al. (2005a) intended to feed Met-deficient diets to cows receiving incremental amounts of Smartamine M, Mepron M85, HMB and HMBi; however, a lack of response in milk protein concentrations may have indicated that the basal diet was bordering on Met-adequacy. With this being the case, Met-bioavailability values were calculated based on changes in plasma Met concentrations. The authors calculated a Met bioavailability for HMBi of approximately 35 ± 6.6% (44 ± 8.3% relative to that of Smartamine M). These values are lower than the values of 42 ± 16.0% (53 ± 20.0% relative to that of Smartamine M) calculated by Schwab et al. (2005b) but the variation is considerably less.

This process may be further complicated when Met analogs are fed. The metabolism of HMB is different than DL-Met (Lobley et al. 2001). Once HMB is absorbed, a portion of it bypasses the liver and is taken up by peripheral tissues for anabolic uses with only the kidney being a major exporter of Met synthesized from HMB de novo. If this is the case, it would exacerbate the problem of generating linear

increases in plasma Met concentrations when incremental amounts of Met analogs such as HMBi are fed. The question which has not been properly addressed is whether the Met status of the basal diet affects the metabolism of Met analogs. Schwab et al. (2005a,b) intended to feed Met-deficient diets, but Schwab et al. (2005a) may have failed to make the diets sufficiently Met-deficient as indicated by the lack of response in milk protein concentrations. However, because the diet was not intentionally made to be Met-adequate, this explanation remains to be only speculative and the full effect of a Met-adequate diet on HMB metabolism remains unanswered.

The AUC technique also has challenges and limitations. One challenge is determining whether pulse-dosing large amounts of Met analogs or Met products into the rumen, such as the 50 g Met equivalent amount of Graulet et al. (2005), saturates the enzyme system which results in non-physiological conditions. As previously discussed, DL-Met and HMB are not metabolized similarly. As a result, pulse-dosing large amounts of Met analogs or Met products may be metabolized differently than feeding smaller amounts in a TMR, thus limiting a direct comparison between the two techniques. A definitive benefit of using the AUC technique is the ability to generate definitive responses in plasma Met concentrations. Pulse-dosing large amounts of Met analogs or Met products is an effective method for increasing plasma Met concentrations (Koenig et al., 1999; Graulet et al., 2005).

Calculating bioavailability values for different Met products brings into question the accuracy and precision of the methodology. As demonstrated by Graulet et al. (2005), the AUC technique is precise. These researchers calculated a bioavailability value of approximately 48 ± 2.1% for HMBi (71% relative to that of Smartamine M). On

the contrary, the precision of using changes in milk protein concentrations for HMBi is much lower with a bioavailability value of 42 ± 16.0% as calculated by Schwab et al. (2005b). Using changes in plasma Met concentrations has a greater precision compared to using changes in milk protein concentrations but it is still less than the AUC method when calculating bioavailability values and has a bioavailability value of approximately 35 ± 6.6% as calculated by Schwab et al (2005a).

CONCLUSIONS

Using changes in milk protein concentrations is the easiest and most farm-friendly method for determining the effectiveness of an Met product for delivering absorbable Met to lactating dairy cows. It is easily obtainable and does not require invasive blood collection; however, it lacks precision. However, this method is dependent upon the basal diet being deficient in Met. The AUC method is very precise and based on the research conducted to date, it generates similar values as using changes in milk protein concentrations and changes in plasma Met concentrations which would indicates that it is also accurate. However, it requires intensive blood sampling and relies on pulse-dosing Met analogs or Met products which is not similar to the manner in which the products would be fed on a production dairy facility. It also does not provide any indication of whether the product is increasing milk protein synthesis which is the ultimate indicator of its' effectiveness as deemed by dairy nutritionists and dairy producers. Given the differences in metabolism between Met analogs and Met products, it has not been ascertained if pulse-dosing Met analogs, as opposed to feeding them in a TMR, further

affects the metabolism of the product. Using changes in plasma Met concentrations in response to feeding incremental amounts of Met analogs or Met products is more precise than using changes in milk protein concentrations but is less precise than the AUC method. However, using changes in plasma Met concentrations is a more practical approach in terms of comparability with the amount and feeding method of the Met product to a commercial dairy facility compared to the AUC method. But, it does not permit for changes in milk protein production to be observed, assuming a Met-adequate diet is fed.

From a research standpoint, it is most important to develop a methodology which will yield both accurate and precise bioavailability calculations which are easily replicated or reproduced in a variety of research settings to determine the amount of absorbable Met or Met precursor. From an on-farm, production standpoint, it is an increase in milk protein yields which will ultimately indicate the utility of Met analogs or Met products. However, it is also important to note that having a high Met bioavailability value is not a prerequisite for increasing milk protein production. The overall diet must be balanced for both Met and Lys is changes in milk protein production are to be expected.

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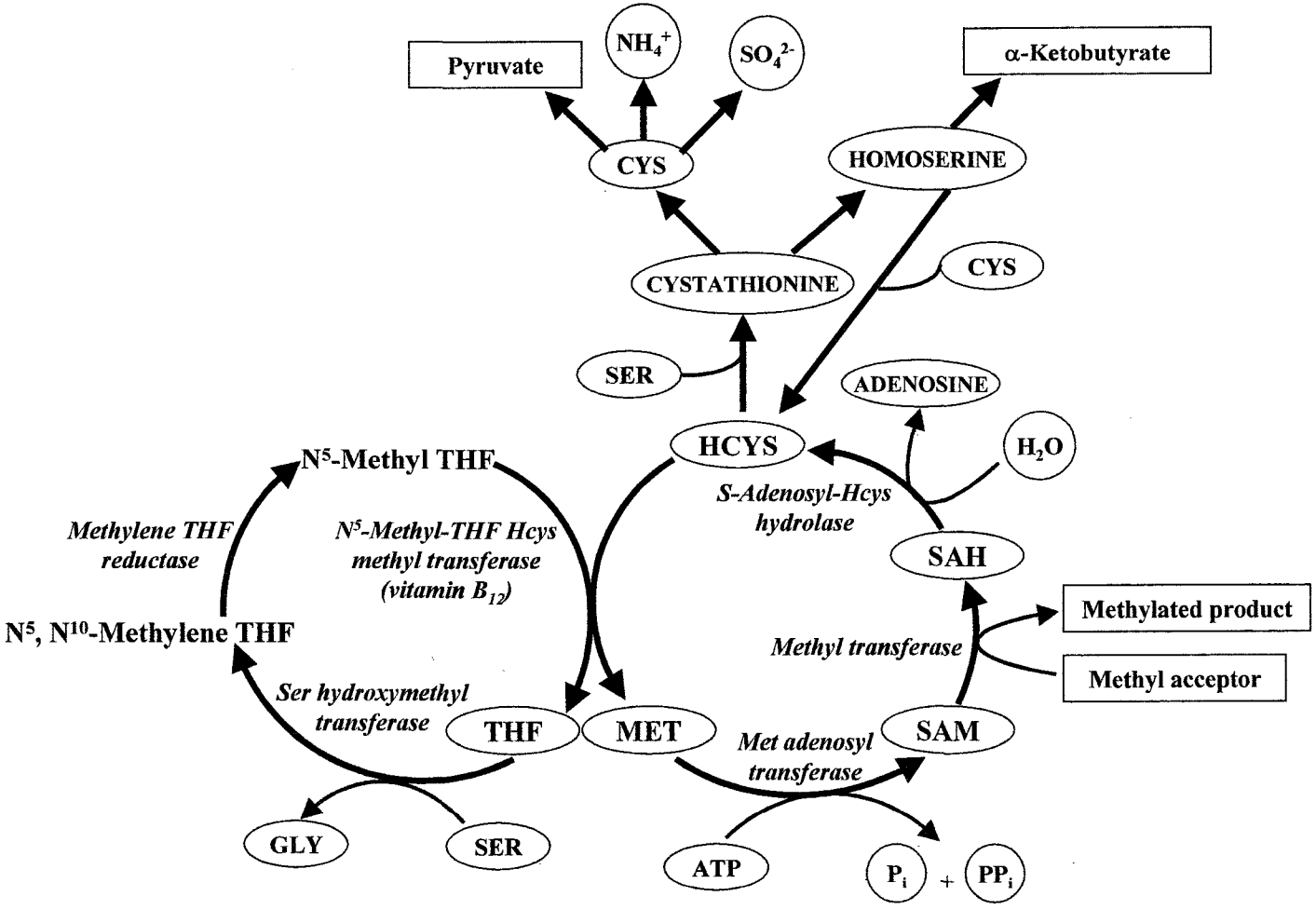
Wester, T. J., M. Vázquez-Añón, D. Parker, J. Dibner, A. G. Calder, and G. E. Lobley. 2000b. Synthesis of methionine (Met) from 2-hydroxy-4-methylthio butanoic acid (HMB) in growing lambs. J. Dairy Sci. 83(Suppl. 1):269.(Abstr.)

Figure 1.1. Metabolic pathways for methionine, cysteine, and homocysteine.



Adapted from McKee and McKee, 1999

CHAPTER II

EFFECTS OF PROVIDING TWO FORMS OF SUPPLEMENTAL METHIONINE TO PERIPARTURIENT HOLSTEIN DAIRY COWS ON FEED INTAKE AND LACTATIONAL PERFORMANCE

ABSTRACT

Eighteen primiparous and 42 multiparous Holstein cows were blocked according to parity and expected calving date and assigned randomly to one of three dietary treatments: 1) basal diet (negative control), 2) basal diet plus 2-hydroxy-4-methylthio butanoic acid isopropyl ester (**HMBi**; hereafter, referred to as, MetaSmart), or 3) basal diet plus rumen protected Met in the form of Smartamine M. Treatments were initiated 21 d before expected calving and continued through 140 d postpartum. Diets were similar in ingredient and chemical composition except for the content of Met in metabolizable protein (**MP**). HMBi and Smartamine M were added to the basal diet in amounts needed to achieve a 3.0:1 ratio of Lys to Met in MP as predicted by the NRC (2001) model. It was assumed that 50% of the HMB in dietary HMBi is absorbed and converted to metabolizable Met and that 80% of the Met in Smartamine M is absorbed. Prepartum dry matter intake (**DMI**) (13.5 kg/d), body weight (**BW**) (687 kg), and body condition score (**BCS**) (3.81), and postpartum milk yield (42.0 kg/d), milk fat yield (1549 g/d), milk fat content (3.66%), milk true protein yield (1192 g/d), and milk urea nitrogen (**MUN**) content (12.9 mg/dl) were not different among treatments. Postpartum DMI and BCS score were greater and the ratio of milk/DMI and milk N/feed N were less for cows

fed MetaSmart than for cows fed the control and Smartamine M diets (22.9 vs. 22.0 and 21.4 kg/d, $P = 0.01$; 3.37 vs. 3.26 and 3.28, $P = 0.09$; 1.92 vs. 2.00 and 1.98, $P = 0.09$; 0.32 vs. 0.33 and 0.34, $P = 0.003$, respectively). Milk protein content ($P < 0.001$) was greater for Smartamine M (2.87%) and HMBi (2.81%) than for control (2.72%). Concentrations of Met and Met+Cys in total plasma amino acids were different among treatments with values for Smartamine M being the highest followed by HMBi and control (2.10, 1.43, and 1.15%, $P < 0.001$; and 3.92, 3.12, and 2.73%, $P < 0.001$, respectively). The results indicated that both HMBi and Smartamine M are effective in providing metabolizable Met, but clarification of their relative contributions to metabolizable Met is still needed.

(**Key words:** methionine, methionine analog, dairy cow)

Abbreviation key: EAA = essential amino acid, **3.5% FCM** = 3.5% fat corrected milk, **HMB** = 2-hydroxy-4-methylthio butanoic acid, **HMBi** = 2-hydroxy-4-methylthio butanoic acid isopropyl ester, **MP** = metabolizable protein.

INTRODUCTION

The ability of a dairy cow to utilize MP for protein synthesis is dependent upon how well the essential amino acid (**EAA**) profile in MP meets the EAA requirements of the animal as well as by the total amount of EAA in MP (NRC, 2001). Research has indicated that Met is often a limiting amino acid for ruminants fed corn silage or haylage based diets such as those typically fed in the United States (Schwab et al., 1992; NRC, 2001). Increasing postruminal supplies of Met to the small intestine has been shown to

be effective in increasing protein synthesis in the mammary gland (Guinard and Rulquin, 1995; Pisulewski et al., 1996). Research has indicated that milk protein content is sensitive to changes in the adequacy of Met in MP (NRC, 2001); therefore, increasing the concentration of Met in MP may lead to increased milk protein production. Another benefit of using rumen protected Met to improve the profile of EAA in MP is that the overall amount of RUP in the diet may be reduced (NRC, 2001). Reducing the amount of dietary N may result in an overall reduction in the amount of N excreted into the environment. Given the environmental concerns and regulations related to food production in the US, this benefit may be valuable and useful.

Supplemental sources of Met have been developed in an effort to increase postruminal supplies of Met. These sources include ruminally protected Met, Met hydroxy analogs such as 2-hydroxy-4-methylthio butanoic acid (**HMB**), and more recently, the isopropyl ester of HMB, MetaSmart. The latter has been shown to increase plasma Met concentrations more than HMB (Graulet et al., 2005; Robert et al., 2003). Although results have been variable, studies have demonstrated that feeding HMB may increase milk (Piepenbrink et al., 2004; Rode et al., 1998) and fat yields (Patton et al., 1970; Rode et al., 1997), but not protein concentrations.

Lysine and Met have been shown to be the two most often limiting AA for milk protein production in lactating dairy cows (NRC, 2001). The NRC (2001) suggests that the optimal ratio of Lys to Met in MP to ensure that neither AA is limiting milk protein production responses is 3.0:1. The objective of this study was to compare the effects on animal performance of feeding Smartamine M and MetaSmart in amounts that result in a predicted 3.0:1 ratio of Lys to Met in MP to prepartum and early lactation dairy cows fed

a Met-deficient diet. We hypothesized that both products would increase milk protein production. However, because approximately 50% of the HMB in MetaSmart appears to be available for use by rumen microorganisms (Schwab et al., 2005a,b), we further hypothesized that a greater response in milk fat production may be observed for cows fed MetaSmart.

MATERIALS AND METHODS

Experimental Design and Treatments

Eighteen primiparous and 42 multiparous Holstein cows were assigned to a completely randomized block design and were blocked according to age (primiparous or multiparous) and expected date of calving. Cows were assigned randomly within parity to one of three dietary treatments: 1) basal diet (negative control: no supplemental Met and Met deficient), 2) basal diet plus MetaSmart (Adisseo USA, Antony, France), or 3) basal diet plus Smartamine M (Adisseo USA, Alpharetta, GA) beginning 21 d before expected calving date and continued on their respective treatments through 140 d postpartum. All cows received the same prepartum (Table 1) and postpartum (Table 2) Met-deficient basal diets.

Preparation and Feeding of Dietary Treatments

Smartamine M and MetaSmart were supplied by Adisseo USA, Inc. (Alpharetta, GA). MetaSmart was supplied as a dry powder consisting of 30% of active ingredient on sepiolite which was used as a carrier. Adjusted Met supplementation levels, based on

Met equivalency of Smartamine M or MetaSmart, were added to the prepartum and postpartum diets of each cow to result in predicted concentrations of Lys and Met in metabolizable protein (**MP**) of 3.0:1 according to the NRC (2001) diet evaluation (Tables 2.1-2.4). The final treatment mixtures consisted of laboratory weighed mixtures of the test products. The amount of Smartamine M and MetaSmart in the final diet treatment mixtures, as weighed for each cow for each feeding, varied according to: (1) test product (due to differences in Met equivalency), (2) treatment level, and (3) DMI.

It was assumed that MetaSmart contained no less than 70% HMB and that 50% of the HMB in MetaSmart was absorbed and converted to metabolizable Met. It was also assumed that Smartamine M contained 75% Met and that 80% of the Met was absorbed (Rulquin and Kowalczyk, 2003).

Management of Cows

Cows were housed in a naturally ventilated tie-stall barn, fed individually, and milked in a milking parlor equipped with automatic take-offs and milk meters. Prepartum cows were fed twice daily, once during the morning feeding (approximately 0700 h) and once during the noon feeding (approximately 1400 h).

All procedures related to animal care were conducted with approval of the University of New Hampshire Institutional Animal Care and Use Committee (IACUC # 010803). The first four blocks of cows were injected every 14 d with bST (Monsanto Company, St. Louis, MO) beginning 84 d after calving. The remaining sixteen blocks of cows were not injected with bST due to changes in the Fairchild Dairy Teaching and Research Center standard operating procedure.

Lactating cows were fed and milked three times each day at 8-h intervals. Weather permitting; lactating cows were exercised for 15-20 min before the second milking in a dry lot. Body weight and BCS (scale of 1-5, with 1 being emaciated and 5 being obese; Wildman et al., 1982), were recorded each week beginning 21 d before expected calving date and continued through 140 d postpartum. Body condition score was originally intended to be evaluated by three independent scorers, however, BCS from one scorer were not included in the final statistical analyses based on the results of a statistical outlier test (SAS, version 8.2).

The basal diet (Tables 2.1-2.2) was fed as a TMR and prepared by weighing each ingredient and mixing in a mobile tumble drum mixer (Data Ranger; American Calan, Inc., Northwood, NH). Treatments were top-dressed onto the TMR of each cow and were fed 20% of their total daily feed allotment and treatment at 0600 h, 50% at 1400 h, and 30% at 2100 h for ad libitum feed intake using fresh feed at each feeding. Feed offered was adjusted daily to achieve 5-10% orts. Orts were collected and weighed daily at 1130 h so that DMI could be calculated daily.

Feed Sampling and Analysis

All feed ingredients were sampled 14 d before the start of the experiment and analyzed for DM (60°C in forced air oven for 4 h for silages and NIR AOAC 991.03 for grains and hay and residual moisture on silages), CP (AOAC 990.03; Leco App. Note 203-821-146), ADF (AOAC 973.18), NDF (Van Soest et al., 1991) using wet chemistry by Dairy One Forage Testing Laboratory (Ithaca, NY) to assist in determining the final formulation of the diets. Thereafter, silages were sampled daily during the a.m. feeding

and analyzed for DM using a microwave oven according to the procedure of the National Forage Testing Association (Undersander, 2004). Total mixed ration samples were taken at each feeding each day from Sunday 1200 h through Friday 0600 h and composited (Sunday 1200 h through Wednesday 0600 h, and Wednesday 1200 h through Friday 0600 h) and evaluated for DM and particle size using the Penn State Particle Size Separator (Heinrichs, 1996). Ort samples were collected at 1100 h Monday through Friday and composited (Monday through Wednesday and Thursday through Friday) for DM analysis and particle size.

Each month, concentrate feeds and silages were sampled on 2 consecutive days, every other week, composited as collected, and a portion of the composited feed was analyzes for CP (AOAC 990.03; Leco App. Note 203-821-146), ADF (AOAC 973.18), NDF (Van Soest et al., 1991) using wet chemistry by Dairy One Forage Testing Laboratory (Ithaca, NY) for ration adjustment. A composite sample of each of the forages and grains at the conclusion of the study was sent for analysis of DM (60°C in forced air oven for 4 h for silages and NIR AOAC 991.03 for grains and hay and residual moisture on silages), CP (AOAC 990.03; Leco App. Note 203-821-146), ADF (AOAC 973.18), NDF (Van Soest et al., 1991), ADICP (AOAC 2001.11; Foss App. Note AN 300), NDICP (AOAC 2001.11; Foss App. Note AN 300), NE_L, NFC (100 - (CP + NDF + ash + ether extract), lignin (AOAC 973.18), starch (YSI 2700 SELECT Biochemistry Analyzer, YSI App. Note 319), sugar (Hall et al., 1999), fat (Tecator Soxtec System HT6, FOSS Tecator App. Note AN301; Subnote AN 3414), ash (AOAC 942.05) and minerals (Thermo Jarrell Ash IRIS Advantage Inductively Coupled Plasma Radial Spectrometer)

using wet chemistry by Dairy One Forage Testing Laboratory (Ithaca, NY; www.dairyone.com/forage/procedures).

A portion of the composited forage and grain samples were also dried to approximately 90% DM in a forced air oven at 55°C (VWR Scientific, West Chester, PA), allowed to equilibrate for 2-4 h to room temperature, and ground to pass through a 1-mm screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) for analysis of AA at the conclusion of the study. A composite sample of each of the ground forage and grain samples were sent for analysis of AA using a Beckman system 6300 High Performance Amino Acid Analyzer (Beckman-Coulter Instruments, CA) by the Experimental Station Chemical Laboratories, University of Missouri-Columbia (Columbia, MO).

Milk Sampling and Analysis

Milk samples were collected from each cow during all 3 milkings 1 d each week from d-4 postpartum through 140 d postpartum. A milk sample was not taken until cows reached a minimum of 4 DIM. Samples were refrigerated until composited by milk weight for the sample date. Samples were preserved with 2-bromo-2-nitropropane-1,3-diol (1 tablet per 40 ml of milk). Samples were analyzed for CP, true protein, fat, lactose, and MUN by mid-infrared spectrophotometric analysis with a Foss 4000 (Foss North America, Eden Prairie, MN) (Dairy One Cooperative, Inc., Ithaca, NY).

Blood Sampling and Analysis

Blood samples were drawn at 112 and 119 DIM, 2 h after the 0600 h feeding via venipuncture of the coccygeal vein into 10 ml evacuated tubes containing sodium heparin

(Becton Dickinson and Co., Rutherford, NJ). Samples were immediately placed in an ice bath and centrifuged within 45 min at 3300 x *g* for 20 min at 5°C. An aliquot of 4 ml of plasma was deproteinized (four volumes of plasma was vortexed with one volume of 15% sulfosalicylic acid) and then placed in the centrifuge for 10 min at 5°C prior to centrifuging at 3300 x *g* for 20 min at 5°C. The supernatant was collected and 0.25-ml aliquots were placed into Nunc CryoTube vials (Nalge Nunc International, Denmark) and stored at -80°C until analyzed for plasma free AA using a Beckman system 6300 High Performance Amino Acid Analyzer (Beckman-Coulter Instruments, CA) by the Experimental Station Chemical Laboratories, University of Missouri-Columbia (Columbia, MO). Between the 112 and 119 d samplings, the deproteinized plasma samples taken at 112 d were stored at -20°C until composited with the 119-d deproteinized samples. Plasma AA concentrations (μg/ml) were adjusted to account for the use of 15% sulfosalicylic acid which was used in a 1:4 ratio with plasma to deproteinize the original plasma sample.

Statistical Methods

Production and intake data were analyzed using the MIXED procedure of SAS [Version 8.2 (2001); (SAS Institute, Inc., Cary, NC)] according to the following model:

$$Y_{ijklm} = \mu + T_i + P_j + TP_{ij} + B_k + C_{ijkl} + W_m + TW_{im} + TPW_{ijm} + e_{ijklm}$$

where,

Y_{ijklm} is the dependent, continuous variable,

μ is the overall mean,

T_i is the fixed effect of the ith treatment (i = 1, 2, 3),

P_j is the fixed effect of the jth parity (i = 1, 2),

TP_{ij} is the fixed effect of the ith treatment by jth parity,

B_k is the random effect of the kth block (k = 1,...20),

C_{ijl} is the random effect of the lth cow within the ith treatment, within the jth parity, and within the kth block (l = 1,..., n_{ijk}),

W_m is the fixed effect of the mth week of experiment (m = 1,...20),

TW_{im} is the fixed effect of the ith treatment by mth week of experiment interaction,

TPW_{ijm} is the fixed effect of the ith treatment by jth parity by mth week of experiment interaction, and

e_{ijklm} is the residual error.

In this model, the random effect of cow within treatment subclasses is used as the error term for the effect of treatments. Residual errors, which are error within cow across time and represent error from repeated measurements in the experimental unit (cow) were modeled using a first-order autoregressive covariance structure. Autoregressive (1) resulted in the smallest Bayesian information criterion of the three covariate structures tested: autoregressive (1), compound symmetry, and unstructured (Littell et al., 1996). Data for multiparous cows were analyzed with and without covariates: previous 305-d mature equivalent milk yield, milk protein yield and percent, and milk fat yield and percent. Analyzing the data without covariates resulted in similar Bayesian information criterion values; therefore, covariates were not included in the final statistical model.

Degrees of freedom were calculated using the Kenward-Roger option of MIXED (SAS, 2001).

Data for AA concentrations were analyzed using the MIXED procedure of SAS [Version 8.2 (2001); (SAS Institute, Inc., Cary, NC)] according to the following model:

$$Y_{ijklm} = \mu + T_i + P_j + TP_{ij} + B_k + C_{ijkl} + e_{ijkl}$$

where,

Y_{ijklm} is the dependent, continuous variable,

μ is the overall mean,

T_i is the fixed effect of the ith treatment (i = 1, 2, 3),

P_j is the fixed effect of the jth parity (i = 1, 2),

TP_{ij} is the fixed effect of the ith treatment by jth parity,

B_k is the random effect of the kth block (k = 1,...20),

C_{ijl} is the random effect of the lth cow within the ith treatment, within the jth parity, and within the kth block (l = 1,..., n_{ijk}), and

e_{ijkl} is the residual error.

The Univariate Procedure of SAS was used to determine outlier cows. An observation, which was greater than 2.5 standard deviations from the mean for milk production and DMI, was considered an outlier. The results of the outlier analysis indicated that three multiparous cows were outliers due to erratic milk production and DMI; therefore these cows were removed from the final statistical analyses.

Overall treatment differences were examined using least squares means (SAS, Version 8.2 (2001); (SAS Institute, Inc., Cary, NC)].

. Significance was declared at $P \leq 0.10$ and a trend in the data was declared at $P \leq 0.15$. Week and block were included in the final statistical model for all analyses. The DIFF option of was used to test treatment differences among least squares means, and the SLICE option was used to analyze differences among weekly treatment means (SAS, Version 8.2 (2001); (SAS Institute, Inc., Cary, NC)].

RESULTS

Ingredient and Chemical Composition of Diets

The ingredient composition of the prepartum and postpartum diets are shown in Tables 2.1 and 2.2. Prepartum and postpartum basal diets were similar in ingredient and chemical composition among treatments and differed only in their Met content.

The chemical composition of the forages used during the study is shown in Table 2.5. The chemical composition of the consumed prepartum and postpartum diets and the AA composition of the feed ingredients are shown in Tables 2.3, 2.4, and 2.6, respectively. The chemical composition of the consumed diets was determined by analyzing each individual feed ingredient for its chemical composition and then entering the feed analysis results into the NRC (2001) model. Particle size estimation of the mixed mostly grass silage, corn silage, prepartum TMR and orts, and postpartum TMR and orts are shown in Table 2.7. Orts as a percent of DMI averaged 20.8% prepartum and 12.1% postpartum and were not different among treatments (Table 2.7).

Prepartum Dry Matter Intake, Body Weight, and Body Condition Score

The average days on trial prior to calving was 18.8 ± 0.89 d and were not observed to be different among treatments. Prepartum DMI, BW, and BCS were not observed to be different among treatments and averaged 13.5 ± 0.45 kg/d, 687 ± 16.5 kg and 3.81 ± 0.051, respectively (Table 2.8). Prepartum DMI as a percent of BW was not different among treatments and averaged 1.97% (Table 2.8).

Postpartum Dry Matter Intake, Body Weight, and Body Condition Score

A significant effect on postpartum DMI was observed (Table 2.8). Supplementation with MetaSmart resulted in greater DMI compared to control and Smartamine M (22.9 vs. 22.0 and 21.4 ± 0.41 kg/d, respectively); however, supplementation with Smartamine M was not observed to elicit an effect on DMI compared to control. There was a significant parity by treatment interaction observed for postpartum DMI indicating that primiparous and multiparous cows responded differently to dietary treatments (Figure 2.1; Table 2.8). For primiparous cows, DMI was observed to be greater for cows fed MetaSmart (20.7 ± 0.68 kg/d) compared to control (18.4 ± 0.68 kg/d) and Smartamine M (18.2 ± 0.68 kg/d) (Figure 2.1; Table 2.8). For multiparous cows, MetaSmart supplementation did not increase DMI (25.2 ± 0.47 kg/d) compared to control (25.6 ± 0.47 kg/d) or Smartamine M (24.5 ± 0.47 kg/d); however, multiparous cows supplemented with Smartamine M had lower DMI compared to controls.

Dry matter intake was also analyzed weekly. Primiparous cows receiving MetaSmart were observed to consume more feed during weeks 4-7 postpartum (20.5 ± 1.11 kg/d) than did control (17.0 ± 1.11 kg/d) or Smartamine M (16.6 ± 1.11 kg/d) cows

and multiparous cows receiving Smartamine M had lower DMI during weeks 11, 13, and 14 compared to control (25.9 vs. 28.2 ± 0.77 kg/d, respectively).

Differences in overall postpartum BW were not observed to be different among treatments and averaged 578 ± 12.7 kg during the first 140 d postpartum (Table 2.8), but a significant parity by treatment interaction was observed. Body weights for primiparous cows were greater for cows supplemented with MetaSmart (554 ± 20.9 kg) compared to both control (515 ± 20.9 kg) and Smartamine M supplemented cows (512 ± 20.9 kg). Multiparous cows fed the control (644 ± 14.4 kg) treatment had significantly greater BW compared to Smartamine M (620 ± 14.4 kg) with MetaSmart (622 ± 14.4 kg) being intermediate and similar to both control and Smartamine M. Cows supplemented with MetaSmart (3.37 ± 0.053) had greater BCS than both control (3.26 ± 0.053) and Smartamine M supplemented cows (3.28 ± 0.053) (Table 2.8). Postpartum DMI as a percent of BW was not different among treatments and averaged 3.82%

Milk Production and Composition

Milk yield (43.0 ± 0.99 kg/d), milk fat concentration (3.66 ± 0.074%) milk true protein yield (1192 ± 26.8 g/d), lactose yield (2075 ± 45.6 g/d), MUN yield (5.7 ± 0.18 g/d), and MUN concentration (12.9 ± 0.29 mg/dl) were not observed to be significantly different among treatments (Table 2.9). When analyzed on a weekly basis, cows receiving Smartamine M produced less milk during weeks 9-11 and 13 (trend for week 12: $P = 0.11$) compared to control.

Fat corrected milk (**3.5% FCM**) averaged 43.7 ± 0.80 kg/d and was not observed to be different among treatments; however, a parity by treatment interaction was

observed (Table 2.9). Primiparous cows supplemented with MetaSmart had greater yields of 3.5% FCM (39.0 ± 1.32 kg/d) compared to Smartamine M (36.5 ± 1.32 kg/d), while the control treatment (36.9 ± 1.32 kg/d) was similar to both MetaSmart and Smartamine M. Supplementation with MetaSmart and Smartamine M did not increase 3.5% FCM yields compared to control (49.4 and 49.3 vs. 51.3 ± 0.91 kg/d, respectively) in multiparous cows.

Milk fat yield was similar among treatments (Table 2.9); however, a parity by treatment effect was observed. Supplementation with MetaSmart (1382 ± 49.2 g/d) in primiparous cows did not increase milk fat yield compared to control (1320 ± 49.2 g/d) but did increase milk fat yield compared to Smartamine M (1288 ± 49.2 g/d). Smartamine M did not increase milk fat yield compared to control in primiparous cows. Supplementation with MetaSmart (1744 ± 33.6 g/d) and Smartamine M (1757 ± 33.6 g/d) in multiparous cows did not increase milk fat yield compared to control (1806 ± 33.6 g/d) cows. Control cows produced more milk fat than MetaSmart supplemented cows with no difference being observed between control and Smartamine M.

As expected, supplementation with MetaSmart and Smartamine M increased milk protein concentrations similarly compared to control (2.81 and 2.87 vs. 2.72 ± 0.031%, respectively) (Table 2.9).

Feed Efficiency and Nitrogen Conversion

Feed efficiency (kg of milk/kg of DMI) and conversion of feed N to milk N (g of feed N/g of milk N) differed among treatments (Table 2.10). Supplementation with MetaSmart resulted in a lower feed efficiency compared to Smartamine M and control

(1.92 vs. 1.98 and 2.00 ± 0.032, respectively). Smartamine M supplementation improved the conversion of feed N into milk N compared to control and MetaSmart (0.34 vs. 0.33 and 0.32 ± 0.004, respectively).

Energy balance as predicted by the NRC (2001) model was observed to be negative for all treatments for the duration of the study (Table 2.10). When energy balance was calculated by treatment (energy required for milk and maintenance was subtracted from net energy intake), MetaSmart supplementation resulted in the least severe energy deficit and was significantly different than both control and Smartamine M (-2.9 vs. –3.9 and -4.2 ± 0.41 Mcal/d, respectively) (Figure 2.2; Table 2.10).

Plasma Amino Acids

Supplementation with both MetaSmart and Smartamine M increased the concentration of Met in total plasma AA compared to control (1.43 and 2.10 vs. 1.15 ± 0.078%, respectively). However, Smartamine M supplementation resulted in an even greater increase in the concentration of Met in total plasma AA compared to MetaSmart. Concentrations of Met+Cys in total plasma AA were also observed to be different among treatments with Smartamine M being the greatest followed by MetaSmart and control (3.92 vs. 3.12 vs. 2.73 ± 0.102%, respectively). Concentrations of total sulfur AA in total plasma AA were also greatest for Smartamine M followed by MetaSmart and control (5.98 vs. 5.01 vs. 4.25 ± 0.160%, respectively) (Table 2.11).

DISCUSSION

There is limited data available on the effects of providing supplemental Met in the form of HMB (Griel et al., 1968; Rode et al., 1998; Johnson et al., 1999; Phillips et al., 2003; Piepenbrink et al., 2004) or rumen protected Met (Luhman et al., 1997; Socha et al., 2005) when supplemented during the prepartum and postpartum periods and in no cases has MetaSmart been fed to dairy cows during the prepartum period. Socha et al. (2005) observed that feeding Met during the prepartum period did not decrease prepartum DMI during the last week before parturition, however, it tended to decrease postpartum DMI. Cows fed Smartamine M averaged 14.2 kg/d DMI during the week before parturition while control cows and Smartamine ML cows averaged 15.1 and 15.2 kg/d, respectively. While not significant ($P > 0.10$), there was a numerical trend for lower DMI when cows were fed Smartamine M prepartum. To further highlight this point, cows were blocked into six groups of 14 cows each prior to calving and although there were only 3 basal diets fed prior to parturition (one control diet, one Smartamine M diet, one Smartamine ML diet) the animals were still analyzed as six separate groups of cows. The decrease in DMI prior to parturition for Smartamine M compared to control and Smartamine ML (13.9 vs. 15.1 and 15.1 ± 0.7, respectively, and 14.5 vs. 15.0 and 15.3, respectively) still exists.

In the current study, differences in mean prepartum DMI were not observed; however when the data were analyzed by week, prepartum DMI during the last 2 wk before calving for Smartamine M was significantly less than control and MetaSmart, which is in agreement with the trend observed by Socha et al. (2005). Overall dietary CP

content for the prepartum diet averaged 13.8% in the current study compared to 15.6% for the prepartum diet of Socha et al. (2005). Overall flows of MP-Lys and MP-Met were also lower in the current study for Smartamine M compared to Socha et al. (2005) for Smartamine M (82 and 27 g/d vs. 101 and 37 g/d, respectively). When DMI as a percent of BW was analyzed as a means of adjusting for any numerical differences in BW, no differences between treatments were observed. There were also no treatment by week, parity by week, or parity by treatment by week interactions observed. These results indicate that any numerical differences in DMI prepartum were most likely the result of differences in BW between the cows, however, when adjusted for BW, no differences existed.

A parity by treatment interaction was observed for postpartum DMI and the results indicate that primiparous cows on the MetaSmart treatment consumed more feed than either control or Smartamine M and multiparous cows consuming the MetaSmart and control treatments had greater DMI than cows supplemented with Smartamine M (Figure 2.1). When DMI was taken as a percent of BW, no differences existed among treatments and there were no treatment by week, parity by week, or parity by treatment by week interactions observed. These results indicate that the observed differences in DMI were due to numerical differences in BW, but when DMI was adjusted for BW, no effect of treatment existed.

Prepartum and postpartum diets were evaluated using the NRC (2001) model assuming the average chemical composition of the feeds used throughout the duration of the study. The diet was formulated to be adequate in energy and protein, however, according to NRC (2001) model predictions using the results of the study, energy content

of the diet was lower than expected. The NRC (2001) model predicted energy to be limiting by 4.4, 3.6, and 4.6 Mcal/d for the control, MetaSmart, and Smartamine M diets, respectively, and consequently, predicted energy to limit milk production (Figure 2.2). Actual milk yields compared to NRC (2001) predicted NE_L-allowable milk and MP-allowable milk yields were 43.4 vs. 37.0 and 40.2 kg/d, respectively, for control, 43.5 vs. 38.4 and 40.6, respectively, for MetaSmart, and 42.0 vs. 35.4 and 37.0, respectively, for Smartamine M. These results indicate that cows produced more milk than the NRC (2001) model predicted was possible based on supplies of energy and MP. Regardless, the NRC (2001) model still predicted that milk production was limited more by energy than by MP. A possible explanation for this may be attributable to the corn silage used throughout the study which was found to be relatively low in starch and sugar content compared to levels from previous year's corn silage fed at the University of New Hampshire (Table 2.3).

The lower DMI observed for multiparous Smartamine M cows compared to control cows during weeks 11 and 13-14 may help to explain why milk yields for multiparous control cows during weeks 9-11, and 13 (trend for week 12; $P = 0.11$) were greater than multiparous Smartamine M cows. It appears that the lower DMI for cows consuming Smartamine M were accompanied by lower milk yields.

No overall differences between treatments were observed and no parity by treatment interactions were detected for milk yield. However, when the data was analyzed by parity, there was a tendency for a difference among treatments. More specifically, primiparous MetaSmart cows, which consumed more feed than both control and Smartamine M, were observed to have significantly greater BCS but not milk yield.

It is possible that the greater amount of feed consumed was utilized for replenishing body condition and not partitioned towards milk production. When the milk production data for multiparous cows was analyzed separately, control cows, which had greater DMI than Smartamine M cows, also produced more milk (50.9 vs. 48.0 kg/d) even though their BCS was observed to be similar. It is plausible that the greater DMI was utilized for milk production and did not contribute to improving body condition score. A trend ($P = 0.15$) was observed for multiparous control cows to produce more milk than multiparous MetaSmart cows despite having similar DMI and lower BCS. Again, it appears that control animals converted more DMI into milk than animals consuming the MetaSmart diet. In addition, control cows were observed to have greater efficiency at converting feed into milk than did MetaSmart cows. As previously discussed, MetaSmart cows appeared to utilize more feed for replenishing body condition than for milk production. Assuming that all cows were in a negative energy balance during the entire postpartum period, it is difficult to explain why we observed that multiparous control cows were in greater negative energy balance compared to MetaSmart cows and similar to Smartamine M cows but produced more milk.

The effects of HMB on ruminal fermentation have been varied. For example, Deswysen et al. (1991) fed 0 or 5 g/d HMB to young and mature wethers. Significant increases in apparent DM, OM, and CP digestibility in young wethers but observed no effect on these parameters in mature wethers was observed. Using continuous culture fermenters, Vázquez-Añón et al. (1999) reported that HMB did not elicit any changes in rumen fermentation. In a more recent study, Vázquez-Añón et al. (2001) increased the concentration of HMB from 0 to 0.88% of DM and again did not observe any changes in

DM, CP, ADF, or starch and sugar digestibility or VFA concentration. However, they did observe increases in NDF digestibility, ruminal ammonia-N concentration and bacterial protein synthesis with increasing concentration of HMB.

Feeding Met products has been shown to increase milk fat synthesis. Patton et al. (1970) and Polan et al. (1970) observed increases in milk fat production with HMB supplementation and attributed this effect to an increase in fatty acid synthesis in the rumen and an alteration in the fatty acid composition of plasma lipoproteins. An analogous explanation to that proposed by Patton et al. (1970) and Polan et al. (1970) would be that increasing milk fat synthesis with HMB and MetaSmart supplementation may be related to an increase in mammary uptake of triacylglycerols (TAG) as a result of an increase in the secretion of triacylglycerol-rich lipoproteins by the small intestine and/or liver as observed in preruminant calves (Auboiron et al., 1994). While this explanation may be plausible for MetaSmart which has been demonstrated to resist ruminal degradation (Robert et al., 2001), HMB has not been shown to resist ruminal degradation to any appreciable amount (Schwab et al., 2005a,b). As a result, when feeding HMB in a TMR where very little product would be expected to resist ruminal degradation, an alteration in the ruminal ecosystem by HMB may be the route of action for altering milk fat production.

Altering the VFA profile in the rumen may be another method through which HMB can increase milk fat synthesis. Robert et al. (2003) recently demonstrated that the amount of HMB available in MetaSmart for ruminal degradation is sufficient to cause an alteration in VFA pattern of non-lactating dairy cows. Noftsger et al. (2003) observed an

increase in the acetate:propionate ratio from 3.57:1 to 4.22:1 when HMB was added to diets fed to continuous culture fermenters.

We originally hypothesized that feeding MetaSmart would increase milk fat concentration as a result of an increase in the acetate to propionate ratio as well as an increase in the uptake of TAG by the mammary gland for reasons previously discussed. Although there was no overall increase in milk fat concentration or yield with MetaSmart, when the data was analyzed on a week by week basis, a significant increase in milk fat concentration was observed during wk 9, 11, and 12 for multiparous cows fed Smartamine M compared to control. In addition, a parity by treatment interaction in milk fat yield and 3.5% FCM was observed. For primiparous cows, MetaSmart supplementation produced numerically more milk compared to Smartamine M supplementation, which resulted in significantly greater milk fat and 3.5% FCM production for MetaSmart compared to Smartamine M. This effect was not observed in multiparous cows receiving MetaSmart or Smartamine M supplementation. This resulted in a significant difference between control and MetaSmart for 3.5% FCM and milk fat yield and between control and Smartamine M for 3.5% FCM. Despite the evidence previously discussed, and because no ruminal measurements were taken to evaluate ruminal fermentation, it is not possible to determine whether or not MetaSmart altered the ruminal environment in such a way as to promote increased milk fat synthesis.

As predicted, milk protein concentrations were greater for MetaSmart and Smartamine M compared to the control. There was no difference between MetaSmart and Smartamine M; however, there was a numerical trend for Smartamine M to increase milk protein concentration more than MetaSmart (2.87 vs. 2.81, $P = 0.12$). It was

originally assumed that 50% of the HMB in dietary MetaSmart was absorbed and converted to metabolizable Met (Schwab et al., 2005a,b) and that 80% of the Met in Smartamine M was absorbed (Rulquin and Kowalczyk, 2003). Based on these assumptions, MetaSmart and Smartamine M diets were formulated to supply similar amounts of metabolizable Met. However, because a trend existed for Smartamine M to increase milk protein concentration more than MetaSmart, it would appear that the Met-bioavailabilities of the two products may be different than assumed. However, despite this observation, results demonstrate that MetaSmart is effective in providing metabolizable Met based on its ability to increase milk protein concentrations compared to the control diet.

Future research should be conducted to determine a more accurate value for the Met bioavailability of MetaSmart. While the current study did not directly measure changes in plasma Met concentrations, it is possible that evaluating changes in plasma Met concentrations may improve the precision when evaluating the Met contribution of a rumen protected Met product than compared to changes in milk protein concentration. In the current study, Smartamine M supplementation resulted in a larger numerical, but not statistical increase in milk protein concentrations compared to MetaSmart. However, it must be noted that there can be large variation when using changes in milk protein concentrations to estimate the bioavailability of Met products. Therefore, when using changes in milk protein concentrations to compare the efficacy of Met products some precision may be lost. However, one of the objectives of feeding Met products is to increase milk protein concentrations; therefore, using changes in milk protein concentrations may be the most applicable methodology to employ.

In the current study and in previous studies, an assumed 80% bioavailability value for Smartamine M has been used (Schwab et al., 2005a,b). In all research studies at the University of New Hampshire, Smartamine M was top-dressed onto the TMR resulting in no physical damage to the product. However, under typical feeding conditions, the bioavailability of Met from Smartamine M may be less because of disruption of the protective coating via physical abrasion caused by mechanical mixing/feeding equipment such as TMR mixers. A product such as MetaSmart, which comes in a liquid form and is spray-dried onto the feed, does not experience any loss in efficacy due to physical abrasion. Therefore, under typical feeding situations, the effectiveness of MetaSmart as a percentage of Smartamine M may be greater than 54%. This suggests an advantage to using a product such as MetaSmart, which does not lose efficacy due to mechanical mixing/feeding.

Particle size was estimated for forages, TMR, and orts. Heinrichs and Kononoff (2002) suggested that approximately 3-8% of the total TMR remain on the top sieve (19-mm) when using the Penn State Particle Separator or sorting may occur. They also suggested that there should be no more than a 20% difference between the amount of TMR and orts remaining on the top sieve. In the current study, approximately 27% of the prepartum TMR and 22% of the postpartum TMR and approximately 38% of the prepartum orts and 44% of the postpartum orts remained on the top sieve. The difference between the prepartum TMR and orts was within the 20% allowable range; however there was a 22% difference between the postpartum TMR and orts. This difference was attributed to the low quality alfalfa hay that was included in the postpartum diet which cows did appear to sort. Because the basal diet was similar for all treatments, any

negative effects of particle size would have affected all treatment groups the same, therefore the difference was not considered detrimental in this study.

CONCLUSIONS

It is concluded that both MetaSmart and Smartamine M are effective in providing metabolizable Met as indicated by increased milk protein concentrations and plasma sulfur AA concentrations. However, clarification of their relative contributions to metabolizable Met is still needed. Future research should concentrate not only on determining the best methodology for predicting the bioavailability of methionine products, but also on reducing the variation associated with predicting bioavailability of Met analogs and Met products.

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Table 2.1. Ingredient composition of prepartum diets fed to cows receiving two forms of Met.

	% of DM		
Item	Control	MetaSmart	Smartamine M
Alfalfa hay	7.99	8.01	7.99
MMG silage[1]	13.24	13.24	13.21
Corn silage	33.38	33.38	33.33
Soyhulls	12.81	12.79	12.83
Ground corn meal	7.05	7.06	7.07
Ground barley meal	19.21	19.19	19.20
Soybean meal	3.67	3.68	3.69
Urea	0.36	0.37	0.38
Mineral Supplement[2]	2.30	2.28	2.30
MetaSmart[3]	-	0.35	-
Smartamine M[4]	-	-	0.06

[1] MMG silage = mixed mostly grass silage

[2] Contains: 2.50% dried corn distillers grains (carrier), 0.50% oil, 18.50% salt, 23.68% magnesium oxide, 48.85% magnesium sulfate, 1.02% maganous oxide, 1.77% zinc sulfate, 0.02% iron sulfate, 0.75% red iron oxide, 0.50% copper sulfate, 0.02% cobalt carbonate, 0.01% calcium iodate, 0.08% selenium 3%, 0.10% flavoring, 0.17% vitamin A, 0.06% vitamin D, 1.47% vitamin E.). The custom mineral premix contains (percent or amount of custom mineral DM): 294 KIU/kg of vitamin A, 68 KIU/kg vitamin D, 1131 IU/kg vitamin E, 13.5% Ca, 1.1% P, 6.6%Mg, 0.03% K, 0.27% S, 10 ppm Se, 1558 ppm Mn, 1648 ppm Zn, 1055 ppm Fe, 219 ppm Cu, 31 ppm Co, 9.2% Cl, and 6.0% Na.

[3] MetaSmart was supplied as a dry powder containing 30% of active ingredient on a sepiolite carrier. A 70% methionine equivalence for MetaSmart with a bioavailability of 50% was assumed. The quantity of MetaSmart added to the diet was that needed to increase Met in MP from 1.99% (Table 3) to 2.43%.

[4] A 75% methionine equivalence for Smartamine M with a bioavailability of 80% was assumed. The quantity of Smartamine M added to the diet was that needed to increase Met in MP from 1.99% (Table 3) to 2.38%.

Table 2.2. Ingredient composition of the postpartum diets fed to cows receiving two forms of Met.

	% of DM		
Item	Control	MetaSmart	Smartamine M
Alfalfa hay	4.57	4.57	4.57
MMG silage[1]	11.44	11.44	11.44
Corn silage	33.38	33.39	33.38
Soyhulls	7.91	7.91	7.91
Corn meal	15.69	15.69	15.69
Barley grain	11.04	11.04	11.04
Soybean meal	7.73	7.73	7.73
Urea	0.36	0.36	0.36
Blood meal	2.22	2.22	2.22
Megalac	1.81	1.81	1.81
Mineral Supplement[2]	3.84	3.84	3.84
MetaSmart[3]	-	0.541	-
Smartamine M[4]	-	-	0.102

[1] MMG silage = mixed mostly grass silage

[2] Contains: 30.30% sodium sesquicarbonate, 30.30% calcium carbonate, 15.15% salt, 10.91% magnesium oxide, 4.85% monosodium phosphate, 3.03% trace mineral and vitamin premix, 4.24% yeast culture (Diamond V XP, Diamond Mills, Inc., Cedar Rapids, IA), and 1.21% MTB100 (Alltech, Inc., Nicholasville, KY). The custom mineral premix contains (percent or amount of custom mineral DM): 294 KIU/kg of vitamin A, 68 KIU/kg vitamin D, 1131 IU/kg vitamin E, 13.5% Ca, 1.1% P, 6.6%Mg, 0.03% K, 0.27% S, 10 ppm Se, 1558 ppm Mn, 1648 ppm Zn, 1055 ppm Fe, 219 ppm Cu, 31 ppm Co, 9.2% Cl, and 6.0% Na.

[3] MetaSmart was supplied as a dry powder containing 30% of active ingredient on a sepiolite carrier. A 70% methionine equivalence for MetaSmart with a bioavailability of 50% was assumed. The quantity of MetaSmart added to the diet was that needed to increase Met in MP from 1.79% (Table 4) to 2.30%.

[4] A 75% methionine equivalence for Smartamine M with a bioavailability of 80% was assumed. The quantity of Smartamine M added to the diet was that needed to increase Met in MP from 1.79% (Table 4) to 2.33%.

Table 2.3. Chemical composition and NRC (2001) evaluation of prepartum diets fed to cows receiving two forms of Met[1].

Item	Control	MetaSmart	Smartamine M
NDF, % DM	38.7	38.7	38.7
ADF, % DM	24.4	24.4	24.3
RDP, % DM	10.1	10.2	10.2
RUP, % DM	3.6	3.6	3.6
CP, % DM	13.7	13.8	13.8
Energy balance, Mcal/d	8.6	8.4	7.5
RDP balance, g/d	-8	-3	-1
RUP balance, g/d	431	432	381
MP supplied, g/d	1196	1195	1133
MP balance, g/d	325	326	288
Lys, g/d	87	87	82
Met, g/d	24	29	27
His, g/d	26	26	25
Lys, % of MP	7.26	7.28	7.24
Met, % of MP	1.99	2.43	2.38
His, % of MP	2.20	2.18	2.21
Lys/Met in MP	3.6/1	3.0/1	3.0/1

[1] NRC evaluation of diet was based upon final production data and feed analyses of study.

Table 2.4. Chemical composition and NRC (2001) evaluation of postpartum diets fed to cows receiving two forms of Met[1].

Item	Control	MetaSmart	Smartamine M
NDF, % DM	32.9	32.9	32.9
ADF, % DM	20.4	20.4	20.4
RDP, % DM	10.0	10.0	10.0
RUP, % DM	6.3	6.4	6.4
CP, % DM	16.3	16.4	16.4
Energy balance, Mcal/d	-4.4	-3.6	-4.6
RDP balance, g/d	23	24	21
RUP balance, g/d	-160	-136	-250
MP supplied, g/d	2412	2525	2360
MP balance, g/d	-129	-107	-199
Lys, g/d	168	174	164
Met, g/d	43	58	55
His, g/d	64	66	62
Lys, % of MP	6.96	6.89	6.95
Met, % of MP	1.79	2.30	2.33
His, % of MP	2.64	2.61	2.63
Lys/Met in MP	3.9/1	3.0/1	3.0/1

[1] NRC evaluation of diet was based upon final results of study.

Table 2.5. Chemical composition of the forages fed to cows receiving two forms of Met.

	Alfalfa hay	MMG silage[1]	Corn silage
Chemical, % DM			
DM	91.8	28.0	30.2
CP	16.2	16.5	7.9
Sol. CP, % CP	43.0	58.3	56.7
NE_L, Mcal/kg	1.3	1.4	1.6
ADF	35.3	39.9	26.6
NDF	45.7	57.2	45.1
NFC[2]	29.2	20.1	38.9
NSC[3]	-	-	33.3
Starch[4]	-	-	30.3
Sugar[5]	-	-	3.0
Ca	1.7	0.8	0.2
P	0.2	0.3	0.2
Mg	0.2	0.2	0.1
K	2.3	2.7	1.4
Na	0.07	0.04	0.01
S	0.2	0.2	0.1
Fe, ppm	127.0	324.0	243.3
Zn, ppm	17.0	27.3	20.3
Cu, ppm	7.0	13.5	6.5
Mn, ppm	22.5	57.0	15.5
Mo, ppm	1.6	1.6	1.0

[1] MMG silage = mixed mostly grass silage

[2] Nonfibrous carbohydrate = 100 - (CP + NDF + ash + ether extract).

[3] Nonstructural carbohydrate = starch + sugar.

[4] Starch was analyzed by Dairy One Forage Testing Laboratory using a YSI 2700 SELECT Biochemistry Analyzer - YSI Incorporated, Application Note Number 319.

[5] Sugar was analyzed by Dairy One Forage Testing Laboratory according to the procedure described in Hall et al. (1999). J. Sci. Food Agric. 79:2079-2086.

Table 2.6. Amino acid composition of feed ingredients fed to cows receiving two forms of Met.

AA[1]	Corn silage	Haycrop silage	Alfalfa hay	Corn meal	Barley meal	Soyhulls	Soybean meal	Blood Meal[2]
Arg	0.17	0.43	0.71	0.48	0.65	0.56	3.75	3.85
His	0.13	0.24	0.33	0.37	0.29	0.30	1.41	6.66
Ile	0.25	0.66	0.64	0.31	0.43	0.43	2.36	0.32
Leu	0.67	1.20	1.15	1.31	0.89	0.78	4.10	13.03
Lys	0.19	0.59	0.83	0.45	0.46	0.75	3.29	9.57
Met	0.12	0.23	0.24	0.21	0.22	0.14	0.74	1.23
Phe	0.28	0.68	0.76	0.57	0.62	0.45	2.65	7.27
Thr	0.24	0.53	0.66	0.39	0.43	0.42	1.98	4.17
Trp	0.04	0.09	0.23	0.10	0.13	0.09	0.66	1.99
Val	0.36	0.88	0.85	0.62	0.64	0.53	2.57	8.85
Ala	0.62	1.32	0.80	0.79	0.51	0.50	2.18	7.99
Asp	0.43	1.16	1.85	0.79	0.80	1.08	5.91	10.11
Cys	0.10	0.15	0.22	0.21	0.29	0.21	0.79	0.72
Glu	0.87	1.17	1.50	1.81	2.86	1.34	9.65	8.07
Gly	0.29	0.74	0.73	0.43	0.52	0.95	2.20	4.38
Pro	0.45	0.72	1.14	0.84	1.26	0.63	2.70	3.46
Ser	0.22	0.40	0.61	0.44	0.47	0.55	2.17	4.14
Tyr	0.16	0.33	0.47	0.30	0.34	0.46	1.83	2.56

[1] Percent of ingredient DM.
[2] Ring-dried blood meal.

Table 2.7. Particle size of TMR, orts, and forages used in prepartum and postpartum diets fed to cows receiving two forms of Met.

			Prepartum		Postpartum	
Screen (% retained)	Corn silage	Haycrop silage	TMR	Orts	TMR	Orts
top	14	57	27	38	22	44
middle	68	31	28	29	29	28
bottom	18	12	45	33	49	28

Orts as a % DMI	Control	MetaSmart	Smartamine	SE[1]
n	20	18	19	
prepartum[2]	20.2	21.1	21.1	1.24
postpartum[3]	12.1	11.9	12.2	0.37

[1] Highest standard error of treatment means is shown.
[2] Prepartum = -21 d through calving.
[3] Postpartum = 1 d through 140 d after calving.

Table 2.8. Effects of feeding two forms of Met on least squares means of DMI, BW, and BCS for prepartum and postpartum cows.

					P-value		
Item	Control	MetaSmart	Smartamine M	SE[1]	Treatment	Parity	Parity*trt[2]
n	20	18	19				
Prepartum							
DMI, kg	13.8	13.7	13.0	0.45	NS	<0.0001	NS
BW, kg[3]	689	692	679	16.5	NS	<0.0001	NS
Primiparous	615	650	615	27.0			
Multiparous	763	734	744	19.0			
DMI, % BW	2.00	1.98	1.92	0.076	NS	NS	NS
BCS[3]	3.79	3.85	3.80	0.051	NS	<0.0001	NS
Postpartum							
DMI, kg	22.0[b]	22.9[a]	21.4[b]	0.41	0.01	<0.0001	0.04
Primiparous	18.4[b]	20.7[a]	18.2[b]	0.68			
Multiparous	25.6[a]	25.2[a,b]	24.5[b]	0.47			
BW, kg[4]	580	588	567	12.7	NS	<0.0001	0.06
Primiparous	515[b]	554[a]	512[b]	20.9			
Multiparous	644[a]	622[a,b]	620[b]	14.4			
DMI, % BW	3.79	3.90	3.76	0.065	NS	<0.001	NS
BCS[4]	3.26[b]	3.37[a]	3.28[b]	0.053	0.09	<0.0001	NS

[1] Highest standard error of treatment means is shown.
[2] Parity*trt = parity by treatment.
[3] Prepartum BW and BCS were obtained weekly for each cow during the last 3 wk (21 d) prepartum.
[4] Postpartum BW and BCS were obtained weekly for each cow during the first 20 wk (140 d) postpartum.
[a,b] = Means in same row with different superscripts differ ($P < 0.10$).

Table 2.9. Effects of feeding two forms of Met on least squares means of milk yield, 3.5% FCM, and milk composition.

					P-value		
Item	Control	MetaSmart	Smartamine M	SE[1]	Treatment	Parity	Parity*trt[2]
n	20	18	19				
Milk yield, kg	43.4	43.5	42.0	0.99	NS	<0.0001	NS
3.5% FCM	44.1	44.2	42.9	0.80	NS	<0.0001	0.09
Fat, g/d	1563	1563	1522	29.8	NS	<0.0001	0.10
Protein, g/d[3]	1170	1215	1192	26.8	NS	<0.0001	NS
Lactose, g/d	2095	2106	2025	45.6	NS	<0.0001	NS
MUN, g/d	5.68	5.68	5.68	0.176	NS	<0.0001	NS
Fat, %	3.65	3.65	3.68	0.074	NS	NS	NS
Protein, %[3]	2.72[b]	2.81[a]	2.87[a]	0.031	0.001	NS	NS
Lactose, %	4.84	4.84	4.83	0.018	NS	<0.0001	0.03
MUN, mg/dl	12.7	12.7	13.2	0.29	NS	NS	NS

[1] Highest standard error of treatment means is shown.
[2] Parity*trt = parity by treatment.
[3] Protein = true protein.
[a,b] = Means in same row with different superscripts differ ($P < 0.10$).

Table 2.10. Effects of feeding two forms of Met on postpartum feed efficiency and conversion of feed N into milk N.

					P-value		
Item	Control	MetaSmart	Smartamine M	SE[1]	Treatment	Parity	Parity*trt[2]
n	20	18	19				
Milk/DMI, kg/kg	2.00[a]	1.92[b]	1.98[a]	0.032	0.09	NS	NS
MilkN/feedN[3]	0.33[b]	0.32[b]	0.34[a]	0.004	0.003	NS	NS
Energy balance[4], Mcal/d	-3.85[b]	-2.86[a]	-4.15[b]	0.409	0.03	NS	NS

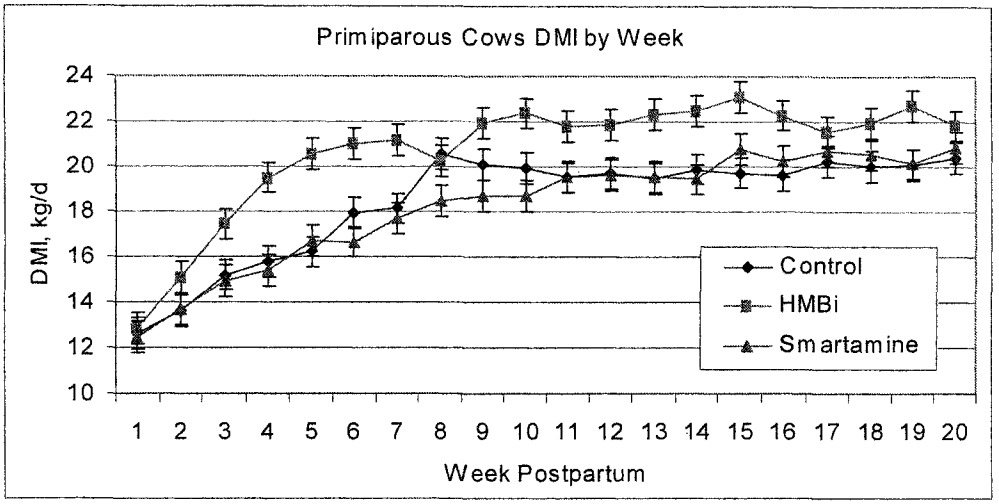
[1] Highest standard error of treatment means is shown.
[2] Parity*trt = parity by treatment.
[3] Milk N yield (kg) per kg N intake.
[4] Calculated for using equations from NRC (2001): Net energy for lactation intake (NE_L predicted from NRC (2001) model x DMI) – [milk energy (0.0929 x fat % + 0.0563 x true protein % + 0.0395 x lactose %) + maintenance energy (0.08 Mcal/kg $BW^{0.75}$)].
[a,b] = Means in same row with different superscripts differ ($P < 0.10$).

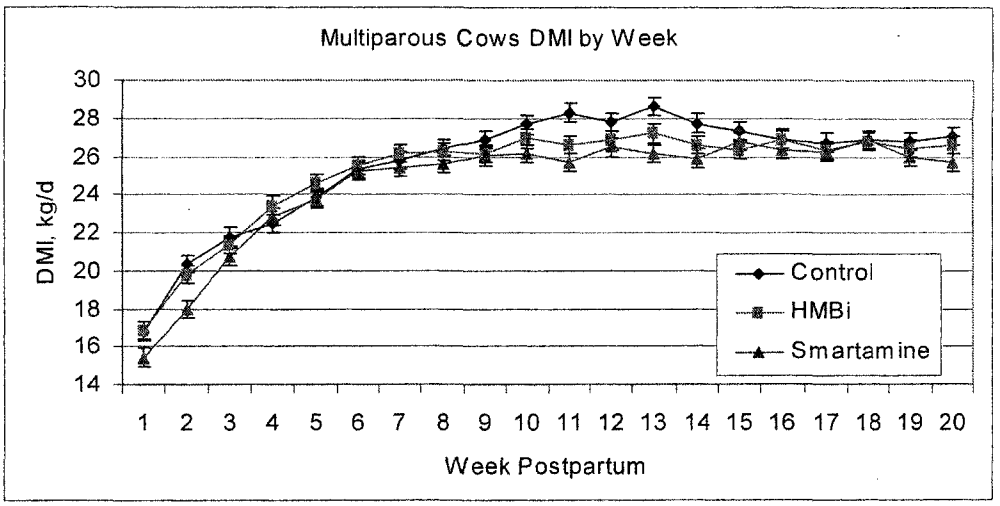
Table 2.11. Effects of feeding two forms of Met on postpartum plasma AA concentrations.

					P-value		
Item	Control	MetaSmart	Smartamine M	SE[1]	Treatment	Parity	Parity*trt[2]
n	14	15	15				
μg/ml[3]							
Met	3.34[c]	4.26[b]	5.96[a]	0.250	<0.0001	NS	NS
Met+Cys	7.97[c]	9.23[b]	11.11[a]	0.353	<0.0001	NS	NS
Total sulfur	12.46[c]	14.87[b]	16.93[a]	0.582	<0.0001	0.05	NS
Total EAA	145.85	140.38	135.84	6.949	NS	NS	NS
TAA	293.18	295.73	284.14	9.025	NS	NS	NS
% of total AA							
Met	1.15[c]	1.43[b]	2.10[a]	0.078	<0.0001	NS	NS
Met+Cys	2.73[c]	3.12[b]	3.92[a]	0.102	<0.0001	NS	NS
Total sulfur	4.25[c]	5.01[b]	5.98[a]	0.160	<0.0001	0.03	NS
Total EAA	49.46	47.16	47.67	1.113	NS	NS	NS

[1] Highest standard error of treatment means is shown.
[2] Parity*trt = parity by treatment.
[3] Samples were analyzed on an as received basis; therefore, AA concentrations shown here have been adjusted to account for the use of 15% sulfosalicylic acid which was used in a 1:4 ratio with plasma to deproteinize the original plasma sample.
[a,b,c] = Means in same row with different superscripts differ ($P < 0.10$).

Figure 2.1. Effects of feeding two forms of Met on postpartum DMI for primiparous, multiparous, and all cows by week.





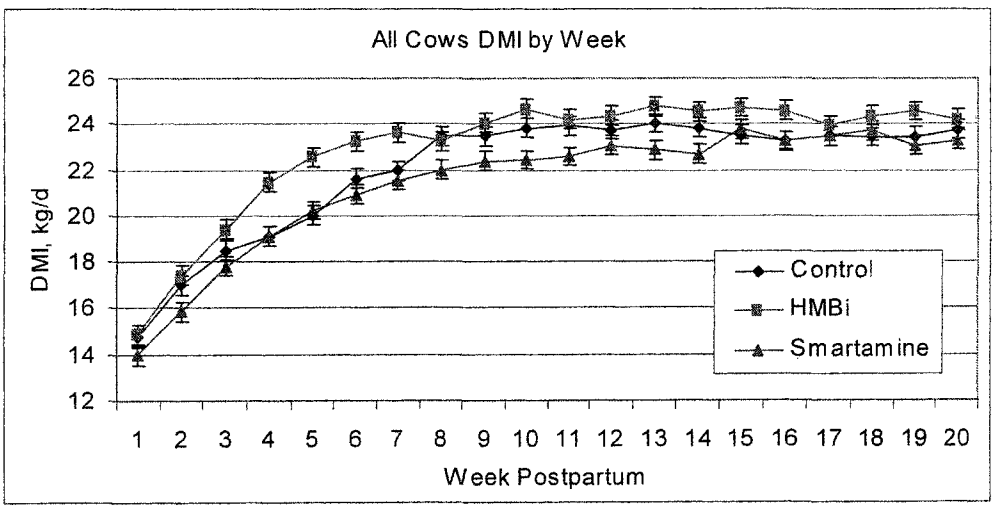
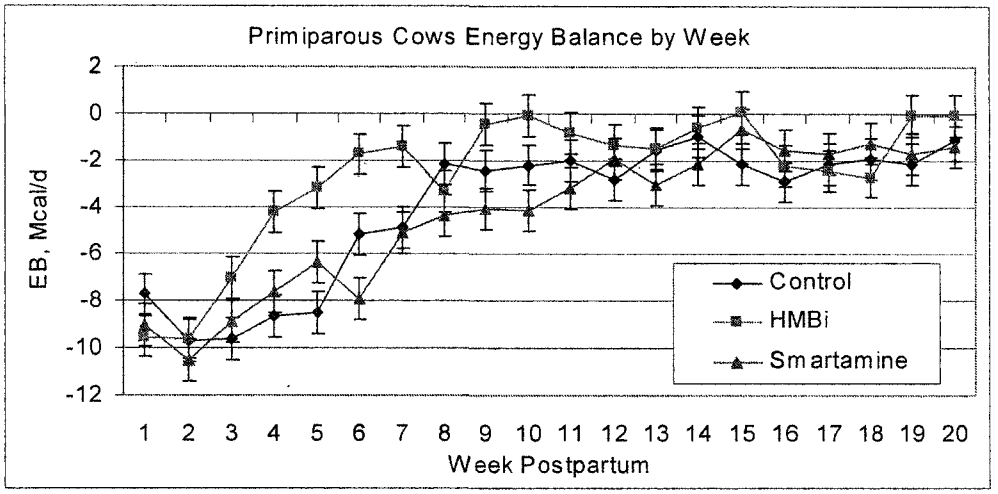
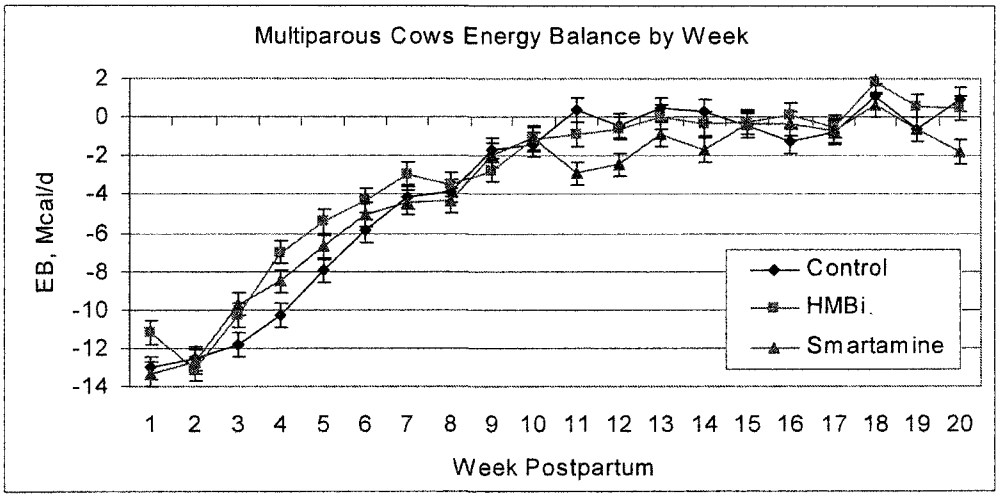
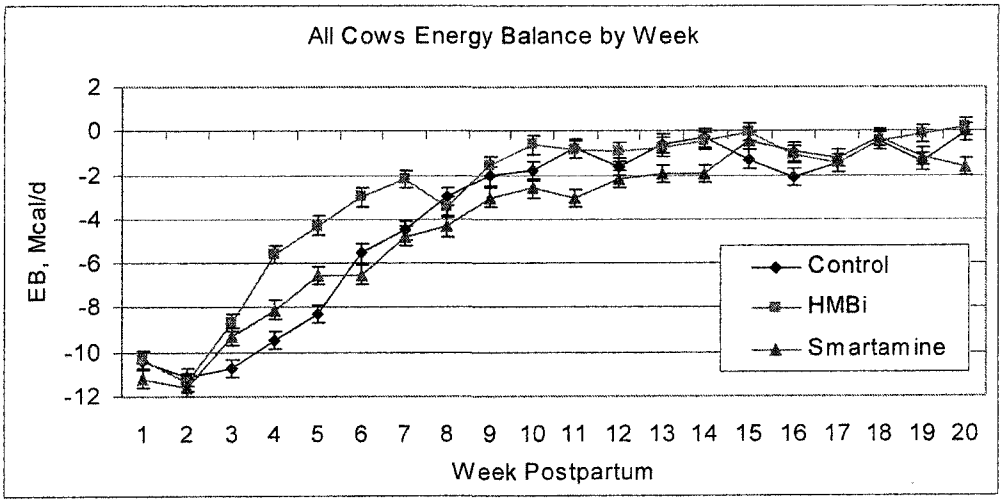


Figure 2.2. Effects of feeding two forms of Met on postpartum energy balance for primiparous, multiparous, and all cows by week.







CHAPTER III

USE OF CHANGES IN PLASMA METHIONINE (MET) CONCENTRATIONS COMPARED TO CHANGES IN MILK PROTEIN CONCENTRATIONS TO COMPARE THE EFFECTIVENESS OF MET PRODUCTS IN THEIR ABILITY TO PROVIDE ABSORBABLE MET TO LACTATING DAIRY COWS FED MET-DEFICIENT OR MET-ADEQUATE DIETS

ABSTRACT

This study was conducted to compare the effects of providing supplemental Met to lactating dairy cows fed Met-deficient or Met-adequate diets on plasma sulfur and milk protein concentrations. This effort was designed to determine the most effective method of measuring the efficacy of Met products. Forty multiparous lactating Holstein dairy cows were used in a replicated randomized complete block split plot 5 x 5 Latin square design with a 2 x 2 x 5 factorial arrangement of treatments and a covariate adjustment of the main plots. The main effects were: 1) Met-adequate or Met-deficient diet, 2) rumen protected Met in the form of Smartamine M or 2-hydroxy-4-methylthio butanoic acid isopropyl ester (**HMBi**; hereafter referred to as, MetaSmart), and 3) five levels (0, 3, 6, 9, or 12 g/d) of Met or Met analog supplementation. The basal diets were formulated to meet NRC (2001) requirements for energy and nutrients and were identical except for predicted Met in MP which were either adequate (i.e., 3.0:1 Lys:Met ratio) or deficient (>3.0:1 Lys/Met ratio) in MP-Met. Smartamine M was used to make the basal diet adequate in MP-Met. Milk protein concentrations for the five respective levels of

supplementation were: Met-adequate MetaSmart (3.05, 3.07, 3.04, 3.06, 3.05 ± 0.044; NS), Met-adequate Smartamine M (3.01, 3.04, 3.06, 3.04, 3.02 ± 0.044; NS), Met-deficient MetaSmart (2.90, 2.92, 2.93, 2.96, 3.01 ± 0.044; linear, $P < 0.05$), and Met-deficient Smartamine M (2.98, 2.96, 3.03, 3.04, 3.03 ± 0.044; linear, $P < 0.05$). Plasma Met concentrations (% of total AA) for the five respective levels of supplementation were: Met-adequate MetaSmart (1.6, 1.6, 1.6, 1.6, 1.6 ± 0.06; NS), Met-adequate Smartamine M (1.6, 1.8, 2.0, 2.2, 2.4 ± 0.06; linear, $P < 0.0001$), Met-deficient MetaSmart (0.8, 0.9, 0.9, 0.9, 1.0 ± 0.06; linear, $P < 0.001$), and Met-deficient Smartamine M (0.8, 1.0, 1.1, 1.3, 1.4 ± 0.06; linear, $P < 0.0001$). Plasma Met+Cys concentrations (% of total AA) for the four respective treatments were:: Met-adequate MetaSmart (3.4, 3.5, 3.5, 3.4, 3.5 ± 0.10; NS), Met-adequate Smartamine M (3.5, 3.7, 3.9, 4.0, 4.3 ± 0.10; linear, $P < 0.0001$), Met-deficient MetaSmart (2.4, 2.4, 2.5, 2.5, 2.6 ± 0.10; linear, $P < 0.0001$), and Met-deficient Smartamine M (2.4, 2.6, 2.8, 3.1, 3.2 ± 0.10; linear, $P < 0.0001$). Plasma total sulfur AA concentrations (% of total AA) for the four respective treatments were:: Met-adequate MetaSmart (5.5, 5.7, 5.8, 5.6, 5.8 ± 0.13; trend, $P = 0.1141$), Met-adequate Smartamine M (5.9, 6.1, 6.4, 6.5, 7.1 ± 0.13; linear, $P < 0.0001$), Met-deficient MetaSmart (4.2, 4.4, 4.5, 4.4, 4.6 ± 0.13; linear, $P < 0.001$), and Met-deficient Smartamine M (4.3, 4.6, 4.9, 5.2, 5.6 ± 0.13; linear, $P < 0.0001$). The Proc Reg procedure of SAS was used to generate regression equations for responses in milk protein concentration, plasma Met, plasma Met+Cys, and plasma total sulfur AA concentrations and concentrations of these AA as a percent of total AA. Bioavailability calculations were calculated by dividing the change in slope of MetaSmart by the change in slope for Smartamine M. Using changes in milk protein concentrations for Met-

deficient cows resulted in a bioavailability calculation of 132 ± 152% which was not considered to be accurate. The bioavailability of MetaSmart relative to that of Smartamine M using plasma Met (% total AA), plasma Met+Cys (% total AA) and plasma total sulfur AA (% total AA) was 27 ± 12.4, 24 ± 16, and 28 ± 9%, respectively. The results of this study demonstrated that using changes in milk protein concentrations to determine Met bioavailability values for MetaSmart relative to that of Smartamine M was not as precise as using changes in plasma sulfur AA concentrations. These results provide further evidence that methionine analogs are metabolized differently than DL-methionine sources and that more research is needed to determine the best response criteria to use for determining the efficacy of methionine analogs.

(**Key words:** methionine, methionine analog, bioavailability)

Abbreviation key: BCAA = branched chain amino acid, **EAA** = essential amino acid, **HMBi** = 2-hydroxy-4-methylthio butanoic acid isopropyl ester, **MMG** = mixed mostly grass, **MP** = metabolizable protein, **NEAA** = non-essential amino acid, **SSA** = sulfur amino acid, **THF** = tetrahydrofolate.

INTRODUCTION

Several methods have been used to determine the effectiveness of Met analogs and protected Met products in providing absorbable Met to lactating dairy cows. However, regardless of the methodology employed, it is essential that the procedure is repeatable and that it provides a true measure of differences in Met availability to the animal.

There are two major categories for determining the bioavailability of Met products. The first category involves using changes in plasma Met concentrations while the second category utilizes changes in milk protein production.

Within the category of using changes in plasma Met concentrations to determine the bioavailability of Met analogs and Met products, two techniques have been developed. The first technique is called area under the curve (**AUC**) (Bach and Stern, 2000; Robert et al., 2001a,b; Robert et al., 2002; Graulet et al., 2005). This technique relies on determining a basal plasma Met concentration for each cow on the study by generating a mean plasma Met concentration from plasma Met samples taken over a period of several hours prior to pulse-dosing Met with the Met analog or the Met product being tested. The basal plasma Met concentrations are then subtracted from the plasma Met concentrations determined for each cow after Met supplementation. The resultant values correspond to increases in plasma Met concentrations resulting from Met supplementation. The second technique utilizes changes in plasma Met concentrations when Met products are added to the diet in incremental amounts (Olley et al., 2004; Schwab et al, 2005b). Methionine bioavailabilities are compared by regressing plasma Met concentrations on levels of supplemental Met. Smartamine M, a protected form of supplemental Met, has been used as the reference Met source and it is assumed that it has a bioavailability of 80% (Rulquin and Kowalczyk, 2003). The slopes of the other Met analogs or Met sources being tested are divided by the slope for Smartamine M to obtain a bioavailability relative to that of Smartamine M.

The second category of determining the bioavailability of Met analogs and Met products utilizes changes in milk protein production (Schwab et al., 2005a) when cows

are fed a diet deficient in Met and supplemented with increasing levels of Met. Changes in milk protein concentrations are regressed on the increasing levels of supplemental Met. Similar to the method of Schwab et al. (2005b), Smartamine M is used as the reference Met source and the slopes of the other Met analogs or Met sources being tested are divided by the slope for Smartamine M to obtain a bioavailability relative to that of Smartamine M.

A summary of the results of Schwab et al. (2005a,b) and Olley et al. (2004) are presented in Tables 3.1, 3.2, and 3.3. These studies indicate that supplementing Met at levels above 10-12 g/d once Met adequacy in the diet has been met does not provide any benefit in determining the bioavailability of Met analogs and Met products using the differences in slope technique described by Schwab et al (2005a,b). It is more beneficial to have all of the Met supplementation levels result in points which lie on the linear part of the slope line because it provides more data points from which to make the bioavailability calculation. Once a plateau in plasma Met concentrations or milk protein production is reached, any points beyond the break-point cannot be used in the bioavailability calculation.

Using changes in milk protein concentrations is the easiest and most farm-friendly method for determining the effectiveness of a Met analog or Met product for delivering absorbable Met to lactating dairy cows. However, this method is dependent upon the basal diet being deficient in Met. In contrast, the AUC method is precise (Graulet et al., 2005) and generates values for MetaSmart similar to using changes in milk protein concentrations (Schwab et al., 2005b) and changes in plasma Met concentrations (Schwab et al., 2005a) which would indicate that it is also accurate. However, it is more

difficult to perform and relies on pulse-dosing Met analogs or Met products which is less similar to the manner in which the products would be fed on a production dairy facility. Using changes in plasma Met concentrations in response to feeding incremental amounts of Met analogs or Met products is more precise than using changes in milk protein concentrations (Schwab et al., 2005b) but is less precise than the AUC method (Graulet et al., 2005). However, using changes in plasma Met concentrations is a more practical approach than the AUC method in terms of comparability with the amounts of product fed and the method of feeding the Met product to dairy cows.

From a research standpoint, it is most important to develop a methodology for comparing the efficacy of Met products which will yield both accurate and precise bioavailability calculations which are easily replicated or reproduced. However, from an on-farm, production standpoint, it is an increase in milk protein concentrations which will ultimately indicate the effectiveness and utility of Met analogs or Met products. We hypothesize that using changes in plasma Met concentrations to measure the ability of Met products to provide absorbable Met to lactating dairy cows fed a Met-adequate diet is more accurate than using changes in milk protein production when lactating dairy cows are fed a Met-deficient diet.

MATERIALS AND METHODS

Experimental Design and Treatments

Forty multiparous lactating Holstein dairy cows were used in a replicated randomized complete block split plot 5 x 5 Latin square design with a 2 x 2 x 5 factorial

arrangement of treatments and a covariate adjustment of the main plots. The main effects were: 1) Met-adequate or Met-deficient diet, 2) rumen protected Met in the form of Smartamine M or 2-hydroxy-4-methylthio butanoic acid isopropyl ester (**HMBi**; hereafter referred to as, MetaSmart), and 3) five levels of Met or Met analog supplementation. Cows were blocked into 5 groups of 4 cows each according to milk production recorded the week before the study. Cows were randomly selected from each block and assigned to one of the four 5 x 5 Latin squares. The 4 squares of cows were randomly assigned to the: 1) Met-deficient diet/MetaSmart treatments, 2) Met-deficient diet/Smartamine M treatments, 3) Met-adequate diet/MetaSmart treatments, and the 4) Met-adequate diet/Smartamine M treatments. The basal diets (Table 3.4) were formulated to meet NRC (2001) model predicted requirements for energy and nutrients and were identical in ingredient composition except for NRC (2001) predicted Met in metabolizable protein (**MP**) which were either adequate (i.e., 3.0:1 Lys:Met ratio) or deficient (>3.0:1 Lys/Met ratio) in MP-Met. Smartamine M was used at 0.088% of diet DM to make the basal diet adequate in Met. The five dietary treatments within squares were 0, 3, 6, 9, or 12 g/d of supplemental metabolizable Met from Smartamine M or the molecular equivalents of HMB from MetaSmart (Table 3.5). Each experimental period was 10 d in length with d 1-6 being used for adjustment to treatment levels and d 7-10 being used for sample collection. Cows were allowed to adapt to the Met-deficient and Met-adequate diets for a period of 28 d before initiation of treatments. Smartamine M and MetaSmart were supplied by Adisseo USA, Inc. MetaSmart was supplied as a dry powder consisting of 60% of active ingredient on a silicate carrier.

Preparation and Feeding of Dietary Treatments

The dietary treatments consisted of laboratory weighed mixtures of the test products. The amounts of test product as weighed for each cow for each feeding varied according to: (1) test product (because of differences in Met equivalency; Table 3.5), (2) supplementation level (Table 3.5), and (4) DMI. A basal level of 22 g/d of Smartamine M per 24.0 kg DMI was added to dietary treatments for the Met adequate cows to create the Met-adequate diets. This amount of Smartamine M was deemed necessary before the start of the experiment to achieve a predicted 3.0:1 ratio of Lys:Met in MP (NRC, 2001).

MetaSmart was supplied as a dry powder containing 60% of active ingredient on a silicate carrier. It was assumed that MetaSmart contains a minimum of 78% HMB, is 95% pure, and that 50% of the HMB in dietary MetaSmart was absorbed and converted to metabolizable Met [57% (60% x 95%) MetaSmart monomer x 77.8% HMB x 50% bioavailability = 22% metabolizable Met]. Smartamine M was assumed to contain 75% Met and that 80% of the Met is absorbed resulting in a metabolizable Met content of 60% (75% x 80%) of the original amount of product supplied (Rulquin and Kowalczyk, 2003).

Management of Cows

All procedures related to animal care were conducted with approval of the University of New Hampshire Institutional Animal Care and Use Committee (IACUC # 040403).

Cows were housed in a naturally ventilated tie-stall barn, fed individually, and milked in a milking parlor equipped with automatic take-offs and milk meters. Lactating

cows were fed and milked three times daily at 8-h intervals. Weather permitting lactating cows were exercised for 15-20 min before each milking in a dry lot. Body weights and BCS were recorded on the same day during the first and last week of the study. Body condition scores were evaluated by two independent scorers and averaged across scorers to obtain one score.

The basal diet (Table 3.4) was fed as a TMR and prepared by weighing each ingredient and mixing in a mobile feed mixer (Super Data Ranger; American Calan, Inc., Northwood, NH). Treatments were top-dressed onto the TMR of each cow and were fed 20% of their total daily feed allotment and treatment at 0600 h, 50% at 1400 h, and 30% at 2100 h for ad libitum feed intake. The basal diet was mixed using fresh feed at each feeding. Feed offered was adjusted daily to achieve 5-10% orts. Orts were collected and weighed daily at 1130 h so that DMI could be calculated daily.

Feed Sampling

All feed ingredients were sampled 14 d before the start of the experiment and 5 d before the end of the 28-d adjustment period and analyzed for DM (60°C in forced air oven for 4 h for silages and NIR AOAC 991.03 for grains and hay and residual moisture on silages), CP (AOAC 990.03; Leco App. Note 203-821-146), ADF (AOAC 973.18), NDF (Van Soest et al., 1991) using wet chemistry by Dairy One Forage Testing Laboratory ((Ithaca, NY; www.dairyone.com/forage/procedures) to assist in determining the final formulation of the diets. Silages were sampled daily for daily DM analysis and for evaluation of particle size twice during each period (d 1-5 and d 6-10). A sample of each forage was retained and used to make period composites. Most feed ingredients

were combined together in a mix at the feed mill and delivered to the research facility; therefore, individual feed ingredients were sampled at the feed mill before delivery. Alfalfa hay was core-sampled at time of delivery and was delivered twice during the study for a total of two core samples. A composite of forages and grains were also analyzed for AA at the conclusion of the study.

Total mixed ration samples were taken daily at each feeding and orts samples were taken daily at 1100 h throughout the Latin square portion of the study and composited twice weekly (d 1-5 and d 6-10 of each period) for analysis of DM and particle size distribution.

Feed Analysis

Analysis of forage DM for ration adjustment was accomplished by using a microwave oven according to the procedure of the National Forage Testing Association (Undersander, 2004). Particle size distribution was evaluated using the Penn State Particle Size Separator (Heinrichs, 1996). A composite sample of each of the forages and grains was sent for analysis of DM (60°C in forced air oven for 4 h for silages and NIR AOAC 991.03 for grains and hay and residual moisture on silages), CP (AOAC 990.03; Leco App. Note 203-821-146), ADF (AOAC 973.18), NDF (Van Soest et al., 1991), ADICP (AOAC 2001.11; Foss App. Note AN 300), NDICP (AOAC 2001.11; Foss App. Note AN 300), NE_L, NFC (100 - (CP + NDF + ash + ether extract), lignin (AOAC 973.18), starch (YSI 2700 SELECT Biochemistry Analyzer, YSI App. Note 319), sugar (Hall et al., 1999), fat (Tecator Soxtec System HT6, FOSS Tecator App. Note AN301; Subnote AN 3414), ash (AOAC 942.05) and minerals (Thermo Jarrell Ash IRIS

Advantage Inductively Coupled Plasma Radial Spectrometer) using wet chemistry by Dairy One Forage Testing Laboratory (Ithaca, NY; www.dairyone.com/forage/procedures).

A portion of the composited forage and grain samples were also dried to approximately 90% DM in a forced air oven at 55°C (VWR Scientific, West Chester, PA), allowed to equilibrate for 2-4 h to room temperature, and ground to pass through a 1-mm screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) for analysis of AA. A composite sample of each of the ground forage and grain samples were sent for analysis of AA using a Beckman system 6300 High Performance Amino Acid Analyzer (Beckman-Coulter Instruments, CA) by the Experimental Station Chemical Laboratories, University of Missouri-Columbia (Columbia, MO).

Milk Sampling and Analysis

Milk yields were recorded at each milking throughout the entire study. On d 7-10 of each experimental period, milk samples were taken during the a.m. and noon milkings and were refrigerated until composited by milk weight according to the respective milking from which the sample was taken during the sampling period. Samples were preserved with 2-bromo-2-nitropropane-1,3-diol (1 tablet per 40 ml of milk). Samples were analyzed for CP, true protein, fat, lactose, and MUN by mid-infrared spectrophotometric analysis with a Foss 4000 (Foss North America, Eden Prairie, MN) (Dairy One Cooperative, Inc., Ithaca, NY).

Blood Sampling and Analysis

Blood samples were taken via venipuncture of the coccygeal vein 3x daily at 2-h intervals after the morning feeding on d 7-10 of each experimental period. Blood samples were taken at 0700, 0900, and 1100 h on d 7 and 9 and at 0800, 1000, and 1200 h on d 8 and 10. Plasma samples were composited within and across days and analyzed in duplicate for AA content. Because cows were fed at 8-h intervals, the sampling protocol was designed to generate average concentrations of plasma AA to eliminate variations in concentration which can occur over time (Graulet et al., 2005). Blood samples were collected via venipuncture of the coccygeal vein into 10-ml evacuated tubes (Vacutainer, Becton Dickinson, Rutherford, NJ) containing sodium heparin. Blood tubes were placed immediately into an ice bath and centrifuged within 45 min at 3300 x *g* for 20 min at 5°C. An aliquot of 4ml of plasma was deproteinized (four volumes of plasma was vortexed with one volume of 15% sulfosalicylic acid) and then placed in the centrifuge for 10 min at 5°C prior to centrifuging at 3300 x *g* for 20 min at 5°C. The supernatant was collected and 0.35-ml aliquots were placed into Nunc CryoTube vials (Nalge Nunc International, Denmark) and stored at -80°C until analyzed for plasma free AA using a Beckman system 6300 High Performance Amino Acid Analyzer (Beckman-Coulter Instruments, CA) by the Experimental Station Chemical Laboratories, University of Missouri-Columbia (Columbia, MO). Plasma AA concentrations (μM) were adjusted to account for the use of 15% sulfosalicylic acid which was used in a 1:4 ratio with plasma to deproteinize the original plasma sample.

Statistical Methods

Production and intake data were analyzed as a randomized complete block split plot 5 x 5 Latin square design with a 2 x 2 x 5 factorial arrangement of treatments with a covariate adjustment of the main plots using the MIXED procedure of SAS [Version 8.2 (2001); (SAS Institute, Inc., Cary, NC)] according to the following model:

$$Y_{ijklm} = \mu + B_i + M_j + S_{kij} + C_{lijk} + L_m + LB_{im} + LM_{jm} + LBM_{ijm} + e_{ijklm}$$

where:

Y_{ijklm} = the continuous, dependent variable,

μ = the overall mean,

B_i = the fixed effect of the i^{th} base diet, i = 1, 2,

M_j = the fixed effect of the j^{th} methionine source, j = 1, 2,

S_{kij} = the random effect of the k^{th} square, k = 1, 2, 3, 4, 5,

C_{lijk} = the random effect of the l^{th} cow on the i^{th} base diet, within the j^{th} methionine source, within the k^{th} square, l = 1, 2, 3, 4, 5,

L_m = the fixed effect of the m^{th} level of methionine supplementation, m = 0, 3, 6, 9, 12,

LB_{im} = the fixed effect of the interaction between the m^{th} level of methionine supplementation and the i^{th} base diet,

LM_{jm} = the fixed effect of the interaction between the m^{th} level of methionine supplementation and the j^{th} methionine source,

LBM_{ijm} = the fixed effect of the interaction between the m^{th} level of methionine supplementation, the j^{th} methionine source, and the i^{th} base diet,

e_{ijklm} = the random residual error.

The effect of level of Met source was partitioned into single degree of freedom orthogonal contrasts within each Met source. Linear, quadratic, cubic and quartic contrasts were fitted within each source. The Univariate Procedure of SAS was used to determine outlier cows [Version 8.2 (2001); (SAS Institute, Inc., Cary, NC)]. An observation, which was greater than 2.5 standard deviations from the mean for milk production, milk protein concentration and milk protein yield was considered an outlier. The results of the outlier analysis indicated that one cow was an outlier due to low milk production and low milk protein yield; therefore, this cow was removed from the final statistical analysis.

The Proc Reg procedure of SAS was used to generate regression equations for determining the slope of the changes in milk protein concentration, plasma Met concentrations (μM and % total AA), plasma Met+Cys concentrations (μM and % total AA), plasma total sulfur AA concentrations (μM and % total AA) [Version 8.2 (2001); (SAS Institute, Inc., Cary, NC)]. The change in slope of AA concentrations for the MetaSmart cows was divided by the change in slope of AA concentrations for the Smartamine M cows to determine a relative bioavailability value for MetaSmart relative to Smartamine M. A bioavailability of 80% was assumed for Smartamine M. Significance was declared at $P \leq 0.05$ and a trend in the data was declared at $P \leq 0.15$.

RESULTS

Chemical Composition of Diets

The chemical and AA composition of the dietary feed ingredients are presented in Tables 3.6 and 3.7, respectively. Because replicates 1 and 2 were not conducted

simultaneously, results of the feed analyses from each replicate were averaged in order to generate an overall chemical composition for each ingredient. However, between periods 3 and 4 of replicate 1, a new mixed mostly grass silage (**MMG**) bunker silo was opened. As a result, the chemical composition of the MMG silage used during periods 1 through 3 of replicate 1 was different from that used during periods 4 and 5 of replicate 1 and all of replicate 2. To account for these differences, the basal diet had to be adjusted, resulting in a higher inclusion rate of alfalfa hay and a lower inclusion rate of MMG silage during periods 4 and 5 of replicate 1 and all of replicate 2 compared to periods 1-3 of replicate 1. The ingredient composition of the basal diets (Table 3.5) was weighted accordingly to reflect the ration adjustment which was made between periods 3 and 4 of replicate 1. A weight of 30% was placed on the inclusion rate of alfalfa hay and MMG silage used during periods 1 through 3 of replicate 1 and a weight of 70% was placed on the inclusion rate of alfalfa hay and MMG silage used during the last 7 periods of the study (periods 4 and 5 of replicate 1 and periods 1 through 5 of replicate 2). Because the same basal diet was used for both the Met-adequate and Met-deficient diets, all cows were affected similarly by the changes to the ingredient composition of the basal diets. The mean concentrations of NDF, ADF, NFC, RDP, RUP and CP in diet DM for the overall study were 34.2, 22.2, 43.7, 9.5, 6.1 and 15.6%, respectively. Forage NDF averaged 24.1% of diet DM and the AA composition of the dietary ingredients (Table 3.7) were similar to those reported in NRC (2001).

Body Weight, Body Condition Score, and Days in Milk

Body condition scores were similar among cows fed Met-adequate and Met-deficient diets and averaged 3.3 ± 0.16 at the beginning of the study and 3.4 ± 0.16 at the end of the study. Body weights were also similar among cows Met-adequate and Met-deficient diets and averaged 662 ± 52.3 kg at the beginning of the study and 674 ± 48.5 at the end of the study.

Because both replicates were not conducted simultaneously, an attempt was made to ensure that cows were similar in lactation number and stage of lactation. Cows in the first replicate averaged 3.4 lactations and were 160 DIM after the 4-wk adjustment period; cows in the second replicate averaged 3.2 lactations and were 159 DIM after the 4-wk adjustment period.

Feed Intake and Milk Yield

Feeding incremental amounts of Smartamine M to cows fed Met-deficient diets and feeding incremental amounts of MetaSmart to cows fed Met-adequate or Met-deficient diets did not affect DMI, milk yield or milk yield to DMI ratios (Table 3.9). Feeding incremental amounts of Smartamine M to cows fed Met-adequate diets did not affect DMI, however, it did result in a linear (P <0.05) decrease in milk yield and milk yield to DMI ratio (Table 3.9).

Milk Composition and Yield of Milk Components

Feeding incremental amounts of MetaSmart to cows fed Met-adequate diets did not affect milk protein concentration (Table 3.10), milk protein yield (Table 3.10), milk

fat concentration (Table 3.10), milk fat yield (Table 3.10), MUN concentration (Table 3.11), lactose concentration (Table 3.11) or lactose yield (Table 3.11). However, feeding incremental amounts of Smartamine M to cows fed Met-adequate diets decreased milk protein yield (linear, $P < 0.01$; Table 3.10), increased milk fat concentration (linear, $P < 0.001$; quadratic, $P < 0.01$; Table 3.10) and decreased lactose yield (linear, $P < 0.01$; Table 3.11). A trend for a quadratic effect was observed for MUN concentration ($P = 0.07$; Table 3.11). Feeding Smartamine M to cows receiving a Met-adequate diet had no effect on milk protein concentrations (Table 3.10), milk fat yield (Table 3.10) or lactose concentrations (Table 3.11).

Feeding MetaSmart to Met-deficient cows increased both milk protein concentration (linear, $P < 0.01$; Table 3.10) and milk protein yield (linear, $P < 0.01$; Table 3.10), but did not affect milk fat concentration (Table 3.10), milk fat yield (Table 3.10), or MUN (Table 3.11). A trend for a cubic effect on lactose concentration ($P = 0.09$; Table 3.11) and a significant cubic effect on lactose yield ($P < 0.05$; Table 3.11) were also observed.

Feeding Smartamine M to cows fed Met-deficient diets increased milk protein concentration ($P < 0.01$; Table 3.10) but did not affect milk protein yield, MUN concentration, or lactose yield. There was an effect of treatment on milk fat concentration (quadratic, $P < 0.05$; Table 3.10), a trend for a quadratic effect ($P = 0.10$) and a significant cubic effect on milk fat yield ($P < 0.05$ Table 3.10), and a trend for a decrease in lactose concentration (linear, $P = 0.0901$; Table 3.11).

Plasma AA Concentrations (μM)

Overall, feeding incremental amounts of MetaSmart to Met-adequate cows did not affect plasma Met concentrations or total plasma AA concentrations (Tables 3.12-3.13). However, there was a trend for a linear increase in Met+Cys concentrations (P = 0.11), which was the result of an increase in plasma Cys concentrations ($P < 0.05$; Table 3.13), and not in plasma Met concentrations. There was also a trend towards an increase in plasma total sulfur AA (**SAA**) concentrations ($P = 0.07$; Table 3.13). In addition to an increase in plasma Cys concentrations, a linear increase in plasma Gly and Ser concentrations was observed ($P < 0.05$; Table 3.13) as well as a trend for a linear increase in Asn ($P = 0.06$; Table 3.13), Asp ($P = 0.10$; Table 3.13), and Pro ($P = 0.06$; Table 3.13) concentrations. These changes were responsible for the observed trend for increased total nonessential AA (**NEAA**) concentrations ($P = 0.06$; Table 3.13).

Feeding incremental amounts of Smartamine M to Met-adequate cows resulted in a highly significant increase in plasma Met concentrations ($P < 0.0001$; Table 3.12). This increase led to a highly significant linear increase in SAA concentrations ($P < 0.0001$; Table 3.13). There was a trend for a quadratic response in total plasma AA concentrations ($P = 0.07$; Table 3.13). This trend was most likely the result of a significant quadratic response in plasma Thr concentrations ($P < 0.05$; Table 3.12) and a trend for a quadratic response in Val concentrations ($P = 0.06$; Table 3.12). These responses most likely are responsible for the trend towards a quadratic response in plasma essential AA (**EAA**) concentrations ($P = 0.09$; Table 3.13) and a trend for a quadratic response in plasma branched chain AA (**BCAA**) concentrations ($P = 0.11$; Table 3.13).

Total plasma AA concentrations decreased linearly when Met-deficient cows were fed incremental amounts of MetaSmart ($P < 0.05$; Table 3.13). This decrease was the result of a significant decrease in plasma NEAA concentrations ($P < 0.05$; Table 3.13). Total plasma EAA concentrations were unaffected by treatment. Decreased plasma concentrations of Gly and Ser ($P < 0.05$; Table 3.13) and a trend for decreased plasma concentrations of Ala ($P = 0.11$; Table 3.13), Asn ($P = 0.06$; Table 3.13), Gln ($P = 0.07$; Table 3.13), and Pro ($P = 0.067$; Table 3.13) were observed.

Feeding incremental amounts of Smartamine M to Met-deficient cows resulted in linear increases in plasma Met concentrations ($P < 0.0001$; Table 3.12), plasma Met+Cys concentrations ($P < 0.0001$; Table 3.12), total plasma SAA concentrations ($P < 0.05$; Table 3.13), and a linear decrease in plasma total NEAA concentrations ($P = 0.09$; Table 3.13). Quadratic responses in plasma total EAA, BCAA, Arg, Ile, Leu, and Lys ($P < 0.05$; Table 3.12) and a trend for quadratic responses in plasma total AA ($P = 0.12$; Table 3.13), Phe ($P = 0.12$; Table 3.12), Thr ($P = 0.06$; Table 3.12), Val ($P = 0.09$; Table 3.12), Asn ($P = 0.08$; Table 3.13), Cys ($P = 0.11$; Table 3.13), and Tyr ($P = 0.12$; Table 3.13) were also observed.

Estimates of "Bioavailability" of Met from MetaSmart

Changes in milk true protein concentration (Figure 3.1; Table 3.16), Met concentration in plasma (μM) (Figure 3.2; Table 3.16), Met concentration in plasma total AA (%) (Figure 3.3; Table 3.16), Met+Cys concentration in plasma (μM) (Figure 3.4; Table 3.16), Met+Cys concentration in plasma total AA (%) (Figure 3.5; Table 3.16), total sulfur AA concentration in plasma (μM) (Figure 3.6; Table 3.16) and total sulfur

AA in plasma total AA (%) (Figure 3.7; Table 3.16) in response to feeding incremental amounts of MetaSmart or Smartamine M were used to estimate the Met-bioavailability of MetaSmart. The slope of the change in each of the aforementioned parameters in response to feeding MetaSmart to Met-adequate or Met-deficient cows was divided by the slope of the change in each of the aforementioned parameters in response to feeding Smartamine M to Met-adequate or Met-deficient cows. Therefore, the bioavailability of MetaSmart was calculated as a percentage of the bioavailability of Smartamine M. If the change in slope was not significant, a bioavailability was not calculated.

The linear slope increase for Met-deficient cows fed incremental amounts of MetaSmart was steeper than the slope for Smartamine M. As a result, the bioavailability of MetaSmart calculated from using changes in milk protein concentrations was greater than 100% (132 ± 152%; Table 3.16). The bioavailability of MetaSmart relative to that of Smartamine M using significant linear increases in the slope of plasma Met (% total AA), plasma Met+Cys (% total AA), and plasma total sulfur AA (% total AA) for cows fed Met-deficient diets were approximately 24 ± 16%, 27 ± 12%, and 28 ± 9%, respectively (Table 3.16). These values equate to overall Met bioavailabilities of 19 ± 13%, 21 ± 10% and 22 ± 7%, respectively.

DISCUSSION

There are many challenges and limitations involved with determining the bioavailability of Met analogs and Met products. Several methods have been developed including using changes in milk protein concentrations in response to feeding Met-deficient diets (Schwab et al., 2005a,b), using changes in plasma Met concentrations in

response to feeding Met-adequate diets (saturation technique; Olley et al., 2004), and using the area under the curve (pulse-dose technique; Graulet et al., 2005). Each method has advantages and disadvantages but none have been universally accepted as the best method for determining bioavailabilities of Met analogs and/or Met products. The most important aspect of developing a methodology for determining bioavailabilities is that it is universally accepted, it has the ability to accurately and precisely predict the bioavailability of both Met analogs and Met products, and that it is capable of being applied to a variety of different research settings.

Using changes in milk protein concentrations has proven to be somewhat problematic as a result of the lack of precision of the bioavailability calculations as well as problems with ensuring that the basal diet is Met-deficient. In a study conducted by Schwab et al. (2005b) using Met-deficient lactating Holstein cows fed incremental levels of Smartamine M, HMB, MetaSmart, or 1/3 HMB, 2/3 MetaSmart bioavailabilities were calculated based on changes in milk protein concentrations. However, HMB did not elicit a linear increase in milk protein, therefore, a bioavailability estimate was not made which is one of the major disadvantages of using this technique. The bioavailability estimates for MetaSmart and 1/3 HMB, 2/3 MetaSmart were approximately 42 ± 16% and 34 ± 25%, respectively, and relative to that of Smartamine M were 53 ± 20% and 43 ± 30%, respectively, assuming an 80% bioavailability for Smartamine M. An obvious problem with this method is the lack of precision in the bioavailability estimate. Despite these disadvantages, a decided advantage of using changes in milk protein concentration is its' applicability. While it may not be the most precise method of determining Met bioavailabilities in terms of variation, it does provide a practical value for dairy

nutritionists and producers who may be looking for responses in actual production parameters as a means by which to gauge the efficacy of Met analogs or Met products.

The saturation technique which utilizes changes in plasma Met concentrations in response to feeding incremental amounts of Met analogs or Met products to Met-adequate cows also has advantages and disadvantages. Although possibly not as dependent on diet as using changes in milk protein concentrations, using changes in plasma Met concentrations is dependent upon the Met status of the basal diet. Theoretically, the more Met-adequate the basal diet is, the more pronounced the response in plasma Met will be. If the Met requirement has been met, then additional supplies of MP-Met will no longer be utilized as rapidly, allowing Met to accumulate in the blood. If the basal diet is not as adequate as was predicted, there may not be a significant linear increase in plasma Met concentrations resulting in the inability to calculate bioavailability values. Schwab et al. (2005a) intended to feed Met-deficient diets to cows receiving incremental amounts of Smartamine M, Mepron M85, HMB and MetaSmart; however, a lack of response in milk protein concentrations may have indicated that the basal diet was bordering on Met-adequacy. With this being the case, Met-bioavailability values were calculated based on changes in plasma Met concentrations. Feeding HMB did not increase plasma Met concentrations, therefore, bioavailability values could not be calculated. The calculated Met bioavailability values for MetaSmart and Mepron M85 were 35 ± 7% and 35 ± 3%, respectively, and 44 ± 8% and 43 ± 4%, respectively, relative to that of Smartamine M (assuming an 80% bioavailability). In regards to MetaSmart, these values are lower than the values of 42 ± 16% and 53 ± 20% calculated by Schwab

et al. (2005b) but the variation is considerably less which is an advantage of using the saturation technique compared to changes in milk protein concentrations.

The AUC technique which utilizes pulse-dosing Met analogs or Met products, is more precise than using changes in milk protein concentrations or the saturation technique. Graulet et al. (2005) demonstrated this in a recent study where MetaSmart, HMB and Smartamine M were pulse-dosed into the rumen of non-lactating Holstein cows in 50 g Met equivalent amounts. The resultant Met bioavailability calculations using the AUC technique and a modified Met digestibility equation of Robert et al. (2001a) determined that the bioavailabilities of MetaSmart, HMB, and Smartamine M were 42 ± 1.3%, 19 ± 1.7% and 64 ± 3.2%, respectively. It is readily apparent that the AUC technique has a high level of precision.

However, a concern of using the AUC technique is whether or not large amounts of Met analogs or Met products when introduced into the rumen behave physiologically similar to feeding smaller incremental amounts in a TMR. The metabolism of HMB has been demonstrated to be different than that of DL-Met (Lobley et al., 2001); thereby raising the question of whether pulse-dosing large amounts of Met analogs or Met products further affects the metabolism beyond the normal metabolic differences between the products. However, a definitive benefit of using the AUC technique is the ability to generate responses in plasma Met concentrations. Pulse-dosing large amounts of Met analogs or Met products is an effective method for increasing plasma Met concentrations as has been demonstrated previously (Koenig et al., 1999; Graulet et al., 2005).

Calculating bioavailability values for different Met products brings into question the accuracy and precision of the methodology. The accuracy being whether the

bioavailability value is correct or not and the precision being the amount of error surrounding the bioavailability value. As demonstrated by Graulet et al. (2005), the AUC technique is precise. On the contrary, the precision of using changes in milk protein concentrations is much lower. Using changes in plasma Met concentrations has a greater precision compared to using changes in milk protein concentrations but it is still less than the AUC method when calculating bioavailability values. Despite these differences in precision, accuracy is the most important aspect of calculating bioavailability values. If the calculated bioavailability value is incorrect (i.e., not accurate), it is irrelevant how precise the value is. Interestingly, the bioavailability calculations are all somewhat similar with the values for changes in milk protein concentrations and AUC both being approximately 42% (Graulet et al., 2005; Schwab et al., 2005b) and the value for changes in plasma Met concentrations being 35% (Schwab et al., 2005a).

In the current study, we hypothesized that using changes in plasma Met concentrations to measure the ability of Met products to provide absorbable Met to lactating dairy cows fed a Met adequate diet is more accurate than using changes in milk protein production when lactating dairy cows are fed a Met deficient diet.

Met Status of the Basal Diets

According to the NRC (2001) evaluation of the basal diets, the Met-deficient diet was deficient in MP-Met by approximately 12 g/d (Table 3.8). As indicated by the lower plasma Met concentrations, the Met-deficient diets were deficient in MP-Met (Table 3.12). Plasma Met concentrations for the 12 g/d supplemental Met level for Met-deficient Smartamine M cows is similar to the 0 g/d supplemental Met level for Met-

adequate Smartamine M cows which indicates that the NRC (2001) was accurate in predicting the flow of MP-Met to the small intestine.

Changes in Milk Protein Production

Cows fed diets that provide adequate amounts of MP-Met would not be expected to respond with higher percentages or yields of milk protein to additional supplies of MP-Met, whereas cows which are deficient in MP-Met would be expected to respond. In a recent study, Schwab et al. (2005a) fed a Met-deficient diet to cows receiving incremental amounts of Smartamine M, HMB, MetaSmart, or Mepron M85. The goal of the study was to utilize the subsequent changes in milk protein concentration as a result of feeding Met-deficient cows incremental amounts of Met products to calculate bioavailability values for HMB, MetaSmart and Mepron M85 relative to that of Smartamine M. However, none of the Met products elicited a response in milk protein concentration, therefore precluding the accurate use of changes in milk protein concentration to calculate bioavailability values. A possible explanation, despite the results of the NRC (2001) evaluation of the diets, would be that the cows were not as deficient in MP-Met as predicted at the beginning of the study.

In the current study, feeding incremental amounts of MetaSmart or Smartamine M to Met-deficient cows increased milk protein concentrations in a linear fashion, thus indicating that the diets were deficient in Met. No response in milk protein concentration was observed for Met-adequate cows fed incremental amounts of Met from MetaSmart or Smartamine M, which indicated that the diets were adequate in Met.

Interestingly, the linear change in slope for MetaSmart was greater than the change in slope for Smartamine M. This resulted in a bioavailability calculation of 132% for MetaSmart relative to that of Smartamine M. The bioavailability calculation for the current study of approximately 132 ± 152% is not in accordance with previous research findings and appears to be inaccurate especially considering the standard error which is larger than the mean bioavailability. These results clearly demonstrate a potential inadequacy of using the slope of the changes in milk protein concentration resulting from feeding incremental amounts of an Met product to Met-deficient cows. Using the slope of one product, such as MetaSmart, and comparing it to the slope of Smartamine M to determine a bioavailability value does not take into consideration the overall level of protein concentration, but only the degree of change in milk protein concentration.

The extent of the response in milk protein concentration to feeding incremental amounts of Met to Met-deficient cows was not as great as expected. Because the study consisted of two replicates which were not conducted simultaneously, differences in ingredient or chemical composition of the diets may have influenced the results. Therefore, for interpretation purposes only, each replicate was analyzed separately to determine if the limited response in milk protein concentration was dependent upon replicate. Body weight, BCS, and DIM were similar between the two replicates; however, two different batches of blood meal were used in replicates 1 and 2. When the diets were evaluated in NRC (2001), the Lys to Met ratio for the Met-deficient diet was greater in the second replicate compared to the first replicate (3.84 vs. 3.69). Flows of digestible Lys in the first replicate were 188 g/d compared to 192 g/d in the second replicate. However, when the milk production data was analyzed by replicate, there was

a clear linear response in milk protein concentration in the first replicate but no response was observed in the second replicate. This was an unexpected finding, given the similar results of the NRC (2001) evaluations of the diets.

Given that the diets in replicate 2 had greater flows of MP-Lys compared to replicate 1 (192 vs. 188 g/d, respectively) according to the NRC (2001) evaluation and that the cows responded differently in milk protein concentration between the two replicates, a physiological manifestation must have arisen as a result of the differences in flows of MP-Lys. Therefore, plasma AA data were analyzed by replicate to determine if a difference in plasma Lys concentrations existed. The results demonstrated a significant difference between the first and second replicates with the first replicate cows having greater plasma Lys concentrations compared to the second replicate cows (98.8 vs. 91.0 ± 2.61 μM; $P < 0.05$). However, plasma Met concentrations were nearly identical for both replicates (34.6 vs. 34.5 ± 1.26 μM; $P = 0.961$). Concentrations of Lys as a percent of total AA for replicate 1 were also greater than replicate 2 (4.78 vs. 4.41 ± 0.127%; $P = 0.04$) while concentrations of plasma Met as a percent of total AA remained similar between the replicates (1.71 ± 0.062%). Plasma Lys concentrations (μM) were 7.9% lower in replicate 2 compared to replicate 1 and as a percent of total AA were 7.7% lower in replicate 2 compared to replicate 1. To help elucidate whether an 8% difference was sufficient enough as to elicit a biological response in milk protein concentrations, we examined the results of a study conducted by McLaughlin et al. (2002) where incremental levels of LysHCl were infused into the duodenum of lactating dairy cows. The study was a balanced split-plot 5 x 5 Latin square with two primiparous and 3 multiparous lactating Holstein cows averaging 69 DIM. Cows were fed a Lys-deficient diet (5.1% Lys and

2.4% Met in MP according to NRC (2001) evaluation) and averaged 24.6 kg/d DMI and 45.4 kg/d milk yield. Lysine (% of total AA) increased in a linear fashion ($P < 0.001$; Figure 3.8). Lysine as a percent of total AA was regressed on measured flows of MP-Lys to the duodenum and a subsequent regression equation was derived to fit the data: $y = 0.0258x - 1.6664$; $R^2 = 0.97$. Using this regression equation, the difference in Lys concentrations as a percent of total AA between replicates 1 and 2 (4.78 vs. 4.41, respectively) indicated that there were 14 g/d more MP-Lys flowing to the small intestine in replicate 1 than in replicate 2. This indicates that the NRC (2001) predicted flows of MP-Lys of 188 and 192 g/d for replicates 1 and 2, respectively, may not be correct. As previously discussed, two different batches of blood meal were used in replicates 1 and 2. The chemical analysis of the blood meal used in replicate 2 indicated that it was of higher quality as a result of having a higher overall CP, RUP, RUP digestibility, AA, and AA digestibility than the blood meal used in replicate 1. However, the physical characteristics of the blood meal may offer a possible explanation as to why it did not appear to supply the amount of MP-Lys predicted by NRC (2001). The blood meal used in replicate 1 was a ring-dried product and had a larger particle size compared to the spray-dried product used in replicate 2 which had a powdery consistency. It is possible that the increased surface area of the spray-dried blood meal allowed for greater microbial attachment which resulted in a higher ruminal degradability thus reducing the RUP content of the CP. These results indicate more research is needed to develop a feed analysis procedure that will better model the effect of particle size on ruminal degradability and digestibility of high RUP feed sources such as spray dried blood meal.

However, the original intention prior to conducting the study was to include both replicates in the final statistical analyses. Therefore, analyzing the data by replicate would not be statistically accurate and as a result, the statistical analyses were conducted based on the combined data from the first and second replicates to maintain the statistical principles which were originally intended. In addition, dietary differences are ubiquitous real-world problems, which can affect the overall efficacy of Met products, especially when using production parameters such as changes in milk protein concentration to gauge their performance. The results of this study reinforce the notion that more detailed feed analyses must be developed which can better characterize the differences and similarities between high RUP feed sources such as blood meal.

MetaSmart vs. Smartamine M Metabolism

Research has demonstrated that not only is supplemental Met utilized differently than Met analogs within species, but it is also utilized differently between ruminants and non-ruminants as well (Baker, 1986; Lobley et al., 2001). In non-ruminants, it has been postulated that HMB is absorbed from the gastrointestinal tract and transported to the liver where it is removed from circulation and transaminated to form Met (Bottje et al., 1998). However, more recent research (Wester et al., 2000a,b) has demonstrated, using sheep, that this pathway may be incorrect, especially in ruminants.

Wester et al. (2003a) infused labeled (1-13C)Met into the jugular vein of growing lambs for 12 h, and then from 3 h onward, infused successive 3 h infusions of 0.55 mg/min saline and 4.4 mg/min HMB into the mesenteric vein. Continuous plasma samples were taken every 20 min during the last 80 min of each infusion. All of the

infused HMB was recovered at the portal vein, with 25% being subsequently extracted by the liver. Results indicated that portal appearance of Met and Cys was unaltered by HMB infusion, however, net splanchnic output of Met decreased while Cys output increased with increasing rates of HMB infusion. Although dietary Met appeared to not be released into peripheral circulation, arterial concentrations of Met increased linearly as well as Met irreversible loss rate. They calculated that the increase in Met irreversible loss rate was equivalent to 40% of the HMB delivered past the liver which was metabolized by peripheral tissues and entered the plasma Met pool. These researchers concluded that the liver does not secrete the Met which was transaminated from the extracted HMB, however, the liver may increase Cys output. It appears from their results that HMB is metabolized extensively by peripheral non-hepatic tissues.

In another study conducted by Wester et al. (2000b), unlabeled HMB was infused into the abomasum of growing lambs for 24 h followed by a 6 h infusion of labeled (1-13C)HMB into the abomasum with another infusion of ($2H^3$)Met into the mesenteric vein. Continuous samples of plasma were taken at 30 min intervals during the last 2 h of infusion from the aorta, portal and hepatic veins. The researchers recovered 75% of the infused HMB at the portal vein with 36% being extracted by the liver. Based on the results of the study, it was concluded that post-splanchnic tissues were involved in the synthesis of Met from HMB and the involvement was in the order of kidney > liver > rumen > jejunum > duodenum > ileum. They also found that the lungs, brain, muscle and skin also synthesized Met from HMB but this accounted for less than 5% of intracellular Met. This study provides further evidence that HMB is being utilized by peripheral tissues to meet their Met requirements. It also provides evidence that any increases in

plasma Met concentrations observed with supplemental MetaSmart feeding (HMB is not assumed to escape ruminal fermentation under normal feeding conditions) is most likely the result of a Met-sparing effect and not necessarily the result of a net export of Met from tissues such as the liver that transaminates HMB into Met.

Effects of Methionine Analogs and Methionine on Plasma Sulfur Amino Acids

Plasma Met and Met+Cys concentrations did not increase when Met-adequate cows were fed incremental amounts of MetaSmart. Therefore, a bioavailability value for MetaSmart based on changes in plasma Met and Met+Cys concentrations and plasma Met and Met+Cys (% total AA) could not be calculated.

An interesting observation was that although feeding incremental amounts of MetaSmart to Met-adequate cows did not increase plasma Met concentrations it did result in significant increases in plasma Cys, Ser, and Gly concentrations. Feeding incremental amounts of MetaSmart to Met-deficient cows led to a trend towards increased plasma Met concentrations ($P = 0.0539$) and linearly decreased Ser and Gly concentrations ($P < 0.05$). Plasma Cys concentrations remained unaffected. Interestingly, this relationship may be explained by examining the metabolic interrelationships of Cys, Ser, and Gly. The carbon skeleton of Cys is derived from Ser and the sulfhydryl group is transferred from Met via Hcys. The enzymes involved in the reaction of Ser to Cys (cystathionine synthase and γ-cystathionase) both require pyridoxal phosphate. Serine is the major source of Gly and can be converted into Gly in a reaction catalyzed by serine hydroxymethyltransferase (also requires pyridoxal phosphate). This reaction results in a chemically reactive formaldehyde group that is transferred to tetrahydrofolate (**THF**)

forming N^5, N^{10}-methylene tetrahydrofolate and intimately linking the reaction with Met metabolism. Perhaps, MetaSmart is metabolized differently under Met-adequate conditions than under Met-deficient conditions. Our results indicate that when Met is limiting, MetaSmart dissociates in the blood to HMB and is then transaminated to Met (McCollum et al., 2000; Robert et al., 2001), which would explain the trend for an increase in plasma Met concentrations observed in the current study. We hypothesize that under Met-adequate conditions, such as in the Met-adequate diet; MetaSmart is absorbed into the blood and dissociates to HMB but is transaminated to Cys instead of Met.

In a recent study conducted by Noftsger et al. (2005), cows were fed either a Met-deficient diet or the same diet supplemented with MetaSmart at 0.13% of diet DM. No changes in plasma EAA or plasma NEAA, except for a significant decrease in plasma Tau concentrations were observed. This supplementation level is similar to the 6 g Met equivalent supplementation rate (0.11% of diet DM) in the current study and is approximately half that of the highest level of MetaSmart supplementation (12 g Met equivalent supplementation rate). No effects on plasma Tau concentrations were observed in the current study for Met-adequate or Met-deficient cows fed incremental amounts of MetaSmart. Concentrations of plasma Cys were not reported; therefore, it is not known whether feeding MetaSmart had an effect on plasma Cys concentrations. These researchers hypothesized that a negative energy balance may have been responsible for the lack of effect of feeding MetaSmart on plasma Met concentrations. Indeed, a negative energy balance may have been culprit considering other researchers have observed increased plasma Met concentrations (Sylvester et al., 2003) or trends for

increased plasma Met concentrations (current study; Schwab et al., 2005a) when Met-deficient cows were fed supplemental amounts of MetaSmart.

Effects on Milk Yield and Dry Matter Intake

A significant linear decrease in milk yield and a subsequent linear decrease in the ratio of milk to DMI were observed for Met-adequate cows receiving incremental amounts of Smartamine M. This effect has not been observed in previous studies conducted in our laboratory (Schwab et al., 2005a,b) where incremental amounts of Smartamine M were fed, however, Socha et al. (2005) and Ordway et al. (2004) observed numerically lower milk yields when Smartamine M was supplemented.

In the study conducted by Socha et al. (2005), Smartamine M was supplemented in a fixed amount of 15 g/d irrespective of DMI. As a result, there may have been some cows with low DMI which may have been receiving excessive amounts of Met, thus causing a depression in DMI and a subsequent depression in milk yield. In fact, these researchers did observe significantly lower DMI when cows were supplemented with Smartamine M, which is most likely, the reason for the numerically lower milk yields. In the study conducted by Ordway et al. (2004), Smartamine M was supplemented in an amount to generate a 3.0:1 ratio of Lys to Met in MP as predicted by NRC (2001) and the amount of product each cow received was respective of DMI. Average flow of digestible Met for Smartamine M cows was 55 g/d which equated to 0.26% of DMI. Dry matter intake was statistically lower compared to supplementation with HMBi and numerically lower compared to the basal control diet. Although not statistically significant, milk yield was numerically lower for cows supplemented with Smartamine M compared to the

HMBi and control diets. In the current study, DMI was not decreased by incremental Smartamine M supplementation in either the Met-adequate or Met-deficient diet. Whether Smartamine M does have a biological effect on lowering DMI and/or milk yield would be dependent upon how much supplemental Smartamine M was offered and if the basal diet was adequate in Met.

The potential effects of sulfur toxicity in ruminants are examined in great detail in a review by Kandylis (1984). A reduction in DMI is a common symptom of excessive Met consumption in cattle and sheep. It also appears that ruminants are less susceptible to excessive HMB consumption than DL-Met but decreased feed intake in lambs has been observed when HMB was included in the diet at 1.2% (Papas et al., 1974). Decreased feed intake in cattle has been observed when DL-Met was infused into the rumen at 2.5% of dietary DMI and at 0.6% of dietary DMI when infused into the abomasum (Satter et al., 1975).

In the current study, the highest level of Met supplementation on the Met adequate diet (12 g/d Met equivalent) resulted in digestible Met flows of 75 g/d according to NRC (2001) which equated to approximately 0.3% of dietary DMI for both MetaSmart and Smartamine M cows. This amount is similar to the amount Ordway et al. (2004) fed to early lactation cows (0.26% of DMI) and is only half of the amount infused by Satter et al. (1975) which resulted in decreased DMI in cattle and therefore, would not be expected to lower DMI. In the current study, lower DMI are not responsible for the linear decrease in milk production as may have been the case in the study by Ordway et al. (2004) where numerically lower DMI were observed. While there is a correlation between excessive DL-Met consumption and decreased DMI which may lead to a

subsequent reduction in milk production, the reason for the decline in milk production in the current study is not discernible.

Bioavailability Calculations

Although there were significant increases in milk protein concentrations, the bioavailability calculation of 132 ± 152% is unlikely correct. As previously discussed, using changes in plasma Met concentrations does not appear to be an effective method for calculating bioavailability values for MetaSmart when cows are fed Met-adequate diets, because in the present study, feeding incremental amounts of MetaSmart to Met-adequate cows did not increase plasma Met+Cys or total sulfur AA concentrations. However, it did result in a significant increase in plasma Cys concentrations, but Smartamine M did not increase plasma Cys concentrations; therefore, a bioavailability value was unable to be calculated for MetaSmart.

Feeding Met-deficient cows incremental amounts of MetaSmart and Smartamine M has been shown to increase plasma Met (% total AA), Met+Cys (% total AA) and plasma total sulfur AA (% of total AA) concentrations (Schwab et al., 2005a). In the current study, feeding both Smartamine M and MetaSmart to Met-deficient cows also increased plasma Met (% total AA), plasma Met+Cys (% total AA) and plasma total sulfur AA (% total AA) which allowed for Met bioavailability values for MetaSmart relative to that of Smartamine M to be calculated. Based on these calculations, using changes in plasma total sulfur AA (% total AA) is the most precise indicator of MetaSmart bioavailability relative to that of Smartamine M compared to changes in

plasma Met (% total AA) and plasma Met+Cys (% total AA) (28 ± 9 vs. 24 ± 16 and.27 ± 12%, respectively).

The bioavailability value of 28 ± 9% relative to that of Smartamine M calculated from changes in plasma sulfur AA (% total AA) equated to an overall Met bioavailability value of 22 ± 7% is considerably lower than previous bioavailability estimates of 42 ± 1% (Graulet et al., 2005),

CONCLUSIONS

Feeding MetaSmart to cows receiving Met-adequate diets does not increase plasma Met concentrations but does increase plasma Cys, Ser, and Gly concentrations. More research must be conducted to gain a better understanding of the differences in metabolism between Met analogs, such as MetaSmart, and Met products, such as Smartamine M. In addition, it appears that MetaSmart is metabolized differently depending upon the Met status of the basal diet. More research should be conducted to determine the mechanism(s) responsible for these metabolic differences. Smartamine M linearly increased plasma Met, Met+Cys, and total sulfur AA when fed to Met-deficient and Met-adequate cows regardless of the Met status of the basal diet. As hypothesized, feeding MetaSmart and Smartamine M to Met-deficient cows increased milk protein concentrations but no increase was observed when fed to Met-adequate cows. The results of this study demonstrate that using changes in milk protein concentrations to determine Met bioavailability values for MetaSmart relative to that of Smartamine M is not as accurate or precise as using changes in plasma sulfur AA concentrations.

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Table 3.1. Summary of plasma AA and milk production results from Schwab et al. (2004a) in which cows were fed incremental amounts of Smartamine M or HMBi[1].

	Met equivalents (g/d)						
	0	10	15	20	25	30	SE
Met, μg/ml[2]							
Smartamine M	2.71	3.19	3.50	4.72	5.14		0.31
HMBi	2.73		2.98	2.86	2.65	3.32	0.31
Met+Cys, μg/ml							
Smartamine M	6.78	7.37	7.57	9.20	9.76		0.63
HMBi	6.93		7.88	6.99	6.63	7.61	0.63
TSAA, μg/ml							
Smartamine M	11.45	11.98	12.73	14.82	15.99		0.93
HMBi	11.70		14.02	11.89	11.12	12.53	0.93
Met, % total AA							
Smartamine M	0.99	1.24	1.41	1.65	1.80		0.07
HMBi	1.00		1.02	1.10	1.07	1.29	0.06
Met+Cys, % total AA							
Smartamine M	2.47	2.87	3.05	3.21	3.41		0.10
HMBi	2.55		2.73	2.70	2.74	3.03	0.12
TSAA, % total AA							
Smartamine M	4.17	4.69	5.20	5.24	5.64		0.19
HMBi	4.34		4.84	4.64	4.64	5.06	0.24
Milk yield[3]							
Smartamine M	43.5	43.6	42.4	43.2	43.3		1.17
HMBi, n = 10	44.8		45.8	44.2	43.4	44.6	1.17
Protein, %							
Smartamine M	2.99	3.08	3.15	3.15	3.13		0.039
HMBi	3.05		3.11	3.16	3.17	3.19	0.039
Protein, g/d							
Smartamine M	1297	1335	1339	1363	1355		35.9
HMBi	1349		1405	1387	1367	1406	35.9

[1] Basal diet was intended to be deficient in Met.
[2] Blood data was based on 5 cows per treatment.
[3] Milk data was based on 10 cows per treatment.

Table 3.2. Summary of plasma AA and milk production results from Schwab et al. (2004b) in which cows were fed incremental amounts of Smartamine M or HMBi [1].

	Met equivalents (g/d)							
	0	5	10	15	17	24	35	SE
Met, μg/ml[2]								
Smartamine M	3.07	3.48	3.63	4.26		4.32		0.31
HMBi	3.00		2.53		3.39	3.41	3.45	0.31
Mepron	2.54		3.09		2.86	2.69	4.08	0.31
Met+Cys, μg/ml								
Smartamine M	7.01	7.22	7.38	8.33		7.99		0.50
HMBi	6.59		5.62		7.52	7.12	6.67	0.50
Mepron	6.13		6.58		6.00	5.65	8.03	0.50
TSAA, μg/ml								
Smartamine M	11.26	11.55	11.76	12.99		13.31		0.88
HMBi	11.08		9.78		13.56	12.24	11.72	0.88
Mepron	10.48		11.07		10.10	10.03	13.37	0.88
Met, % total AA								
Smartamine M	1.19	1.41	1.61	1.74		1.97		0.06
HMBi	1.14		1.16		1.32	1.44	1.63	0.06
Mepron	1.10		1.33		1.35	1.41	1.66	0.06
Met+Cys, % total AA								
Smartamine M	2.76	2.94	3.29	3.47		3.67		0.06
HMBi	2.55		2.66		2.95	3.02	3.17	0.06
Mepron	2.66		2.82		2.87	3.00	3.27	0.06
TSAA, % total AA								
Smartamine M	4.43	4.82	5.33	5.48		6.02		0.15
HMBi	4.24		4.65		5.52	5.13	5.69	0.15
Mepron	4.58		4.77		4.83	5.34	5.46	0.15
Milk yield[3]								
Smartamine M	43.3	42.5	42.7	43.2		41.9		1.16
HMBi	42.6		43.7		42.9	44.0	45.1	1.16
Mepron	42.0		42.3		43.0	43.2	42.3	1.16
Protein, %								
Smartamine M	3.13	3.20	3.20	3.20		3.16		0.037
HMBi	3.12		3.19		3.17	3.15	3.17	0.037
Mepron	3.16		3.21		3.19	3.200	3.23	0.037
Protein, g/d								
Smartamine M	1363	1369	1376	1393		1330		44.0
HMBi	1331		1383		1355	1388	1430	43.5
Mepron	1320		1354		1365	1380	1360	43.6

[1] Basal diet was intended to be deficient in Met.
[2] Blood data was based on 10 cows per treatment.
[3] Milk data was based on 10 cows per treatment.

Table 3.3. Summary of plasma AA and milk production results from Olley et al. (2004) in which cows were fed incremental amounts of Smartamine M, Mepron M85, or Met Plus[1].

	Met equivalents (g/d)					
	0	3	6	9	12	SE
Met, μg/ml[2]						
Smartamine M	5.36	5.34	5.91	6.86	6.44	0.46
Met Plus	5.14	4.83	5.32	5.62	4.71	0.46
Mepron	4.83	6.17	6.04	5.74	6.18	0.46
Met+Cys, μg/ml						
Smartamine M	8.83	8.90	9.56	10.60	10.23	0.58
Met Plus	8.86	8.58	9.08	9.35	8.36	0.58
Mepron	8.77	10.39	10.14	9.82	9.99	0.58
TSAA, μg/ml						
Smartamine M	14.35	14.57	15.44	16.72	16.82	0.79
Met Plus	14.94	15.09	15.71	15.50	14.52	0.79
Mepron	15.82	17.43	17.46	16.98	17.02	0.79
Met, % total AA						
Smartamine M	1.95	2.06	2.23	2.48	2.44	0.133
Met Plus	1.88	1.91	1.99	2.05	1.98	0.146
Mepron	1.81	2.16	2.11	2.03	2.23	0.166
Met+Cys, % total AA						
Smartamine M	3.21	3.42	3.62	3.84	3.89	0.159
Met Plus	3.25	3.40	3.41	3.41	3.49	0.163
Mepron	3.29	3.65	3.57	3.48	3.62	0.184
TSAA, % total AA						
Smartamine M	5.25	5.59	5.84	6.05	6.36	0.246
Met Plus	5.47	5.99	5.91	5.71	6.15	0.284
Mepron	6.01	6.20	6.17	6.03	6.18	0.246
Milk yield[3]						
Smartamine M	35.1	35.3	35.8	36.1	36.4	1.51
Met Plus	36.8	37.1	36.4	35.2	35.2	1.51
Mepron	34.6	34.5	33.4	34.2	37.2	1.51
Protein, %						
Smartamine M	2.70	2.76	2.71	2.77	2.77	0.14
Met Plus	2.99	3.04	3.01	3.07	2.96	0.14
Mepron	2.93	2.89	2.90	2.97	2.95	0.14
Protein, g/d						
Smartamine M	941	982	969	992	1003	44.1
Met Plus	1074	1103	1042	1059	1013	44.1
Mepron	982	961	946	982	1042	44.1

[1] Basal diet was intended to be adequate in Met.
[2] Blood data was based on 5 cows per treatment.
[3] Milk data was based on 5 cows per treatment.

Table 3.4. Ingredient composition of the basal diets of mid-lactation cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	% of DM	
Item	Deficient	Adequate
Alfalfa hay	9.04	9.04
MMG silage	11.42	11.42
Corn silage	28.01	28.01
Ground corn meal	12.25	12.25
Ground barley meal	14.16	14.16
Soyhulls	7.37	7.37
Citrus pulp	2.50	2.50
Soybean meal	9.77	9.77
Blood meal, ring dried	1.21	1.21
Energy Booster 100	1.25	1.25
Minerals[2]	3.04	3.04
Smartamine M	-	0.088

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.

[2] Contains (as-fed basis): 29.95% sodium sesquicarbonate, 13.65% calcium carbonate, 15.25% salt, 12.41% magnesium oxide, 12.41% calcium sulfate, 5.78% monosodium phosphate, 3.09% trace mineral and vitamin premix, 4.60% yeast culture (Diamond V XP, Diamond Mills, Inc., Cedar Rapids, IA), and 1.05% MTB100 (Alltech, Inc., Nicholasville, KY), 1.81% Zinpro 4-plex (Zinpro Corp., Eden Prairie, MN). The trace mineral and vitamin pre mix contains (percent or amount of trace mineral and vitamin premix DM): 292 KIU/kg of vitamin A, 67 KIU/kg vitamin D, 1122 IU/kg vitamin E, 10.5% Ca, 1.3% P, 7.5%Mg, 0.02% K, 2.45% S, 10 ppm Se, 1794 ppm Mn, 2135 ppm Zn, 1104 ppm Fe, 378 ppm Cu, 64 ppm, 9.2% Cl, and 6.0% Na.

Table 3.5. A description of dietary treatments of mid-lactation cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Dietary treatments				
Met supplement	1	2	3	4	5
Met-deficient diets	g/24.0 kg DMI				
Assumed MP-Met provided	0	3	6	9	12
MetaSmart product provided[2]	0	14	27	41	55
Smartamine M provided[3]	0	5	10	15	20
Met-adequate diets					
Assumed MP-Met provided	0	3	6	9	12
MetaSmart product provided[2]	0	14	27	41	55
Smartamine M provided[3]	0	5	10	15	20

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.

[2] MetaSmart was supplied as a dry powder containing 60% of active ingredient on a silicate carrier. It was assumed that MetaSmart contains a minimum of 78% HMB, is 95% pure, and that 50% of the HMB in dietary MetaSmart was absorbed and converted to metabolizable Met [57% (60% x 95%) MetaSmart monomer x 77.8% HMB x 50% bioavailability = 22% metabolizable Met].

[3] It was assumed that Smartamine M contains no less than 75% methionine and that it has a bioavailability of 80%.

Table 3.6. Chemical composition of the dietary feed ingredients fed to mid-lactation cows receiving Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1,2].

Chemical % DM	Alfalfa hay	MMG silage	Corn silage	Corn meal	Barley meal	Soyhulls	Citrus Pulp	Soybean meal	Blood Meal[3]
DM	89.7	32.1	28.7	87.6	87.5	89.9	88.6	89.6	91.8
CP	19.8	12.6	7.8	7.6	11.7	11.0	5.9	54.4	98.5
RUP	-	-	-	-	-	-	-	-	82.3
RUP digestibility	-	-	-	-	-	-	-	-	70.4
ADF	37.3	43.7	26.6	3.9	8.8	47.8	18.6	6.4	-
NDF	45.4	65.9	44.0	8.8	19.4	66.5	21.1	9.5	-
NFC[4]	26.6	12.9	41.3	79.3	66.2	21.0	66.3	32.3	-
NSC[5]	8.0	4.9	34.1	75.1	64.5	3.8	25.7	15.4	-
Starch[6]	0.8	0.7	32.2	71.7	58.5	1.3	0.9	1.4	-
Sugar[7]	7.3	4.3	2.0	3.4	6.1	2.5	24.8	14.1	-
Ash	9.1	7.8	4.3	1.4	2.5	4.6	6.9	7.2	-
Fat	1.8	4.3	3.9	4.0	2.1	1.4	2.3	1.2	-
Ca	1.16	0.52	0.27	0.02	0.09	0.69	2.03	0.41	-
P	0.26	0.29	0.26	0.30	0.46	0.13	0.11	0.91	-
Mg	0.35	0.18	0.13	0.10	0.12	0.22	0.11	0.31	-
K	2.26	2.47	1.36	0.42	0.52	1.46	1.10	2.58	-
Na	0.055	0.046	0.006	0.002	0.010	0.003	0.027	0.005	-
S	0.23	0.18	0.10	0.09	0.13	0.09	0.07	0.39	-
Fe, ppm	181	298	270	32	95	444	72	169	-
Zn, ppm	19	23	19	16	26	33	8	43	-
Cu, ppm	10	9	7	2	4	7	6	16	-
Mn, ppm	29	64	11	4	21	13	5	36	-

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Chemical composition of feed ingredients is based upon feed analyses of composited feeds from replicate 1 and replicate 2.
[3] Ring-dried blood meal.
[4] Nonfibrous carbohydrate = 100 - (CP + NDF + ash + ether extract).
[5] Nonstructural carbohydrate = starch + sugar.
[6] Starch was analyzed by Dairy One Forage Testing Laboratory using a YSI 2700 SELECT Biochemistry Analyzer - YSI Incorporated, Application Note Number 319.
[7] Sugar was analyzed by Dairy One Forage Testing Laboratory according to the procedure described in Hall et al. (1999). J. Sci. Food Agric. 79:2079-2086.

Table 3.7. Amino acid composition of feeds fed to mid-lactation cows receiving Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

AA	Alfalfa hay	MMG silage[2]	Corn silage	Corn meal	Barley meal	Soyhulls	Citrus pulp	Soybean meal	Blood meal[3]
					% of CP				
Arg	5.46	2.78	1.90	5.00	5.37	4.78	4.05	7.40	4.41
His	2.55	1.46	1.42	3.24	2.56	2.74	1.75	2.83	7.29
Ile	5.10	3.64	3.57	3.86	3.84	3.76	3.23	4.59	0.94
Leu	8.53	6.55	8.86	13.35	7.43	6.50	5.87	7.74	13.98
Lys	6.34	3.48	2.35	3.38	3.81	6.63	2.67	6.31	9.78
Met	1.77	1.36	1.87	2.28	1.82	1.15	1.20	1.39	1.16
Phe	5.88	3.99	3.50	5.48	5.45	3.84	4.06	5.19	7.52
Thr	5.33	3.34	2.98	3.86	3.62	3.61	3.19	3.87	4.19
Trp	1.31	0.40	0.55	0.88	0.97	0.62	1.11	1.42	1.88
Val	6.83	4.96	4.85	5.44	5.40	4.56	4.34	4.86	9.75
Ala	6.21	6.45	8.69	8.17	4.43	4.41	4.84	4.54	-
Asp	17.19	6.43	5.26	7.21	6.13	9.07	10.00	11.02	-
Cys	1.50	0.83	1.14	2.39	2.30	1.69	1.16	1.42	-
Glu	10.62	7.40	10.25	19.28	23.54	10.54	8.74	17.42	-
Gly	5.46	4.13	3.91	4.12	4.29	8.60	4.25	4.18	-
Pro	13.43	4.04	6.13	9.56	11.01	5.23	11.45	5.02	-
Ser	4.84	2.78	2.63	4.49	3.93	4.83	3.46	4.12	-
Tyr	3.37	2.06	1.70	3.53	2.84	4.06	2.17	3.59	-
Tau	0.36	0.49	0.94	1.51	0.88	0.87	1.57	0.08	-
Hyp	1.05	0.40	0.42	0.29	0.09	4.71	1.66	0.13	-
Orn	0.36	0.91	0.80	0.22	0.28	0.27	0.37	0.16	-

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] MMG = mixed mostly grass.
[3] Ring-dried blood meal

Table 3.8. Chemical composition and NRC (2001) evaluation of the basal diets of mid-lactation cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1,2,3].

Item	Deficient	Adequate
NDF, % DM	34.2	34.2
ADF, % DM	22.2	22.2
RDP, % DM	9.5	9.5
RUP, % DM	6.1	6.1
CP, % DM	15.6	15.6
Energy balance, Mcal/d	2.9	1.8
RDP balance, g/d	-72	-66
RUP balance, g/d	-4	-118
MP supplied, g/d	2814	2732
MP balance, g/d	-1	-90
Lys, g/d	190	185
Met, g/d	51	63
His, g/d	67	65
Lys, % of MP	6.75	6.77
Met, % of MP	1.81	2.31
His, % of MP	2.38	2.38
Lys/Met in MP	3.73:1	2.94:1

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] NRC (2001) evaluation of diet was based upon an assumed DMI of 24.0 kg/d.
[3] Chemical composition of individual feed ingredients used in NRC (2001) evaluation was based upon feed analyses of composited feeds from replicate 1 and replicate 2.

Table 3.9. Dry matter intake (DMI), milk yield, and milk yield to DMI ratios of mid-lactation cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
DMI, kg/d									
Adequate MetaSmart	25.9	26.1	25.6	26.5	26.1	0.70	0.4407	1.0000	0.4542
Adequate Smartamine M	26.5	26.6	26.1	26.7	26.4	0.70	0.8691	0.8355	0.6940
Deficient MetaSmart	27.2	26.4	26.8	26.9	26.9	0.70	0.8917	0.1687	0.1711
Deficient Smartamine M	26.6	26.7	26.3	26.4	26.7	0.70	0.8827	0.4045	0.4891
Milk yield, kg/d									
Adequate MetaSmart	41.4	42.4	41.8	41.9	42.0	1.43	0.6279	0.5275	0.3161
Adequate Smartamine M	42.4	41.8	41.2	41.8	40.9	1.43	0.0458	0.7588	0.3308
Deficient MetaSmart	43.8	43.1	43.1	44.0	43.1	1.43	0.7764	0.8285	0.0757
Deficient Smartamine M	41.2	41.4	41.1	41.4	41.7	1.43	0.4760	0.6189	0.6577
Milk/DMI, kg/kg									
Adequate MetaSmart	1.59	1.62	1.63	1.58	1.61	0.044	0.8204	0.4062	0.0627
Adequate Smartamine M	1.61	1.58	1.57	1.57	1.54	0.044	0.0203	0.9128	0.4577
Deficient MetaSmart	1.61	1.63	1.62	1.64	1.61	0.044	0.9397	0.2922	0.5964
Deficient Smartamine M	1.55	1.55	1.57	1.57	1.57	0.044	0.4164	0.6670	0.6229

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Highest standard error of treatment means is shown.

Table 3.10. Milk composition and yield of milk components of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Protein, %									
Adequate MetaSmart	3.05	3.07	3.04	3.06	3.05	0.044	0.9166	0.9824	0.9374
Adequate Smartamine M	3.01	3.04	3.06	3.04	3.02	0.044	0.7404	0.1438	0.9506
Deficient MetaSmart	2.90	2.92	2.93	2.96	3.01	0.044	0.0013	0.3651	0.7935
Deficient Smartamine M	2.98	2.96	3.03	3.04	3.03	0.044	0.0140	0.7234	0.1516
Protein, g/d									
Adequate MetaSmart	1267	1301	1272	1293	1274	45.2	0.9203	0.4824	0.7470
Adequate Smartamine M	1272	1265	1263	1248	1188	45.2	0.0098	0.1558	0.4953
Deficient MetaSmart	1252	1231	1264	1335	1308	45.2	0.0015	0.7457	0.0244
Deficient Smartamine M	1225	1232	1238	1248	1251	45.2	0.3150	0.9799	0.9132
Fat, %									
Adequate MetaSmart	3.39	3.39	3.39	3.32	3.33	0.097	0.2019	0.7657	0.5482
Adequate Smartamine M	3.29	3.25	3.32	3.27	3.56	0.097	0.0006	0.0040	0.1652
Deficient MetaSmart	3.30	3.38	3.36	3.33	3.31	0.097	0.7989	0.2755	0.4337
Deficient Smartamine M	3.37	3.35	3.28	3.22	3.39	0.097	0.5745	0.0395	0.0672
Fat, g/d									
Adequate MetaSmart	1405	1440	1418	1404	1395	59.7	0.5105	0.4269	0.4748
Adequate Smartamine M	1384	1354	1368	1335	1398	59.7	0.9258	0.1982	0.5866
Deficient MetaSmart	1421	1427	1443	1493	1438	59.7	0.2482	0.3835	0.1846
Deficient Smartamine M	1384	1394	1342	1314	1396	59.7	0.5201	0.1020	0.0498

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Highest standard error of treatment means is shown.

Table 3.11. Milk composition and yield of milk components of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
MUN									
Adequate MetaSmart	11.8	11.8	11.4	11.7	11.7	0.49	0.7651	0.6168	0.9123
Adequate Smartamine M	11.6	12.5	12.0	12.3	11.7	0.49	0.8940	0.0691	0.6568
Deficient MetaSmart	12.6	12.0	12.1	11.9	12.4	0.49	0.6223	0.1185	0.9916
Deficient Smartamine M	12.9	12.7	12.8	12.9	12.8	0.49	0.9791	0.8908	0.5296
Lactose, %									
Adequate MetaSmart	4.77	4.78	4.74	4.77	4.74	0.044	0.2551	0.9525	0.8547
Adequate Smartamine M	4.78	4.77	4.75	4.75	4.76	0.044	0.4435	0.5607	0.9388
Deficient MetaSmart	4.69	4.70	4.67	4.64	4.69	0.044	0.3391	0.3183	0.0901
Deficient Smartamine M	4.74	4.70	4.69	4.70	4.68	0.044	0.0901	0.4538	0.5451
Lactose, g/d									
Adequate MetaSmart	1987	2037	1986	2026	1985	77.2	0.8700	0.4312	0.8229
Adequate Smartamine M	2027	2001	1971	1963	1883	77.2	0.0017	0.4622	0.5060
Deficient MetaSmart	2039	1993	2033	2099	2048	77.2	0.2003	0.8789	0.0361
Deficient Smartamine M	1952	1967	1924	1935	1934	77.2	0.4938	0.8534	0.6434

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Highest standard error of treatment means is shown.

Table 3.12. Plasma essential AA (EAA) concentrations (μM) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Arg									
Adequate MetaSmart	84.0	82.0	83.1	83.7	83.6	5.00	0.8952	0.6572	0.5431
Adequate Smartamine M	85.6	86.5	86.5	88.4	86.7	5.00	0.5350	0.6761	0.6902
Deficient MetaSmart	92.6	94.6	90.6	91.3	88.3	5.00	0.0700	0.5054	0.7406
Deficient Smartamine M	86.7	89.9	93.4	87.2	87.6	5.00	0.8861	0.0479	0.3289
His									
Adequate MetaSmart	58.7	59.6	61.2	62.6	60.8	2.95	0.1910	0.3935	0.4581
Adequate Smartamine M	58.1	60.0	60.1	59.3	58.2	2.95	0.9470	0.3077	0.7956
Deficient MetaSmart	65.1	63.1	62.3	62.5	61.0	2.95	0.1067	0.7474	0.5888
Deficient Smartamine M	65.0	66.6	62.8	62.3	62.8	2.95	0.1032	0.8747	0.2347
Ile									
Adequate MetaSmart	132.0	124.5	124.7	125.6	124.4	6.70	0.2833	0.3938	0.4508
Adequate Smartamine M	107.8	113.5	111.1	108.6	103.6	6.70	0.3379	0.1909	0.6968
Deficient MetaSmart	125.5	135.8	122.7	132.0	123.5	6.70	0.5499	0.3203	0.6698
Deficient Smartamine M	122.6	130.4	130.7	126.5	118.2	6.70	0.3358	0.0194	0.7866
Leu									
Adequate MetaSmart	160.4	150.3	146.8	148.9	150.6	8.31	0.1789	0.1115	0.6493
Adequate Smartamine M	134.2	141.8	136.8	135.4	129.9	8.31	0.3612	0.2461	0.6106
Deficient MetaSmart	153.7	166.8	150.8	163.0	152.0	8.31	0.6457	0.2727	0.7103
Deficient Smartamine M	152.0	159.5	158.7	153.9	142.8	8.31	0.1220	0.0263	0.8942
Lys									
Adequate MetaSmart	95.3	93.6	95.2	96.5	95.6	4.48	0.6640	0.8910	0.4916
Adequate Smartamine M	94.1	95.7	96.0	95.7	92.5	4.48	0.7106	0.3027	0.8502
Deficient MetaSmart	94.3	97.3	92.7	95.9	91.0	4.48	0.3167	0.3954	0.9480
Deficient Smartamine M	91.8	96.5	99.3	95.9	92.7	4.48	0.8861	0.0204	0.7955

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.

Table 3.12 cont'd. Plasma essential AA (EAA) concentrations (μM) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Met									
Adequate MetaSmart	38.9	38.8	40.5	39.5	40.1	2.11	0.3699	0.7760	0.9773
Adequate Smartamine M	38.0	44.7	48.7	51.9	57.4	2.11	<0.0001	0.4516	0.1796
Deficient MetaSmart	21.1	22.4	21.9	23.8	23.9	2.11	0.0539	0.9850	0.9875
Deficient Smartamine M	20.0	24.8	28.0	32.4	34.3	2.11	<0.0001	0.2768	0.7657
Phe									
Adequate MetaSmart	47.7	46.0	45.0	45.2	45.3	1.49	0.1160	0.2399	0.8082
Adequate Smartamine M	43.1	45.3	44.7	45.5	44.0	1.49	0.5703	0.1619	0.8920
Deficient MetaSmart	46.3	48.8	46.1	47.3	46.6	1.49	0.7903	0.5317	0.3431
Deficient Smartamine M	45.2	46.6	46.4	44.8	43.8	1.49	0.1780	0.1241	0.5026
Thr									
Adequate MetaSmart	112.7	115.1	114.1	115.8	116.3	5.47	0.3835	0.9198	0.8224
Adequate Smartamine M	111.0	115.8	119.5	112.3	108.3	5.47	0.3576	0.0136	0.6523
Deficient MetaSmart	133.0	129.7	123.9	128.1	122.5	5.47	0.0144	0.6233	0.4203
Deficient Smartamine M	119.9	123.0	126.1	121.3	118.2	5.47	0.5850	0.0644	0.8409
Trp									
Adequate MetaSmart	37.1	37.5	37.6	36.6	36.3	1.34	0.2850	0.3819	0.7410
Adequate Smartamine M	36.9	37.8	36.8	36.4	35.9	1.34	0.1607	0.4582	0.4460
Deficient MetaSmart	40.1	39.1	39.1	39.7	38.4	1.34	0.2354	0.9927	0.2120
Deficient Smartamine M	38.4	37.7	38.8	38.1	37.5	1.34	0.5142	0.5984	0.4626
Val									
Adequate MetaSmart	296.6	277.6	277.5	280.2	282.1	15.10	0.3243	0.1637	0.4653
Adequate Smartamine M	247.9	265.4	262.1	253.2	241.2	15.10	0.3684	0.0564	0.5345
Deficient MetaSmart	286.2	304.3	277.1	296.9	279.5	15.10	0.4419	0.4498	0.7642
Deficient Smartamine M	282.3	298.6	290.2	284.4	272.4	15.10	0.2082	0.0916	0.4901

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.

Table 3.12 cont'd. Plasma essential AA (EAA) concentrations (μM) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
EAA									
Adequate MetaSmart	1063.5	1024.8	1025.6	1034.7	1035.1	41.15	0.5492	0.3532	0.5397
Adequate Smartamine M	956.6	1006.4	1002.2	986.8	957.7	41.15	0.8341	0.0862	0.6272
Deficient MetaSmart	1057.9	1101.8	1027.2	1080.6	1026.6	41.15	0.2865	0.4648	0.8863
Deficient Smartamine M	1023.8	1073.7	1074.2	1046.6	1010.3	41.15	0.4903	0.0322	0.6064
BCAA[3]									
Adequate MetaSmart	589.0	552.4	549.0	554.7	557.1	29.39	0.2592	0.1781	0.5019
Adequate Smartamine M	489.9	520.6	509.9	497.3	474.7	29.39	0.3499	0.1124	0.5853
Deficient MetaSmart	565.3	606.9	550.6	592.0	554.9	29.39	0.5127	0.3549	0.7213
Deficient Smartamine M	556.9	588.6	579.5	564.8	533.4	29.39	0.1958	0.0424	0.6573
Met+Cys									
Adequate MetaSmart	82.6	84.8	86.5	85.4	86.5	3.29	0.1073	0.4266	0.6207
Adequate Smartamine M	82.7	89.8	95.0	97.1	102.3	3.29	<0.0001	0.2886	0.3525
Deficient MetaSmart	62.3	62.4	62.4	65.0	65.0	3.29	0.1275	0.6893	0.6257
Deficient Smartamine M	59.0	66.2	71.5	76.7	79.2	3.29	<0.0001	0.1195	0.8739

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.
[3] BCAA = comprised of values for Leu, Lys, and Valine.

Table 3.13. Plasma nonessential AA (NEAA) concentrations (μM) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Ala									
Adequate MetaSmart	299.3	293.4	300.6	311.7	291.1	13.32	0.9358	0.3370	0.0462
Adequate Smartamine M	292.2	291.3	301.2	288.4	284.5	13.32	0.4372	0.3040	0.9361
Deficient MetaSmart	310.8	288.1	293.5	292.7	290.6	13.32	0.1077	0.1845	0.1890
Deficient Smartamine M	287.5	297.8	286.2	290.5	279.9	13.32	0.3063	0.3281	0.7427
Asn									
Adequate MetaSmart	54.0	55.5	56.5	58.9	56.6	2.36	0.0567	0.2456	0.3437
Adequate Smartamine M	52.2	54.1	54.8	52.1	49.6	2.36	0.1281	0.0288	0.7731
Deficient MetaSmart	62.0	60.0	59.9	60.6	57.5	2.36	0.0580	0.7720	0.2078
Deficient Smartamine M	57.3	58.8	59.6	58.3	56.6	2.36	0.5768	0.0795	0.9913
Asp									
Adequate MetaSmart	2.8	2.9	2.7	3.0	3.2	0.26	0.0983	0.2393	0.6914
Adequate Smartamine M	2.6	2.4	2.9	2.6	2.7	0.26	0.5001	0.6710	0.6977
Deficient MetaSmart	3.3	2.9	3.2	3.1	3.0	0.26	0.5184	0.8246	0.2936
Deficient Smartamine M	2.9	2.8	2.7	3.1	2.6	0.26	0.6092	0.5970	0.2033
Cit									
Adequate MetaSmart	90.0	89.8	89.9	87.5	89.6	5.25	0.6655	0.7887	0.5620
Adequate Smartamine M	88.7	94.0	87.6	90.3	91.0	5.25	0.9095	0.9827	0.2043
Deficient MetaSmart	96.9	97.5	98.3	97.5	93.9	5.25	0.4046	0.2352	0.6738
Deficient Smartamine M	99.0	95.2	97.5	94.0	97.2	5.25	0.4979	0.3293	0.9307
Cys									
Adequate MetaSmart	43.7	46.0	46.0	45.9	46.4	1.96	0.0484	0.2358	0.3118
Adequate Smartamine M	44.7	45.1	46.3	45.2	44.9	1.96	0.8306	0.2769	0.9757
Deficient MetaSmart	41.2	40.0	40.5	41.2	41.1	1.96	0.6790	0.4464	0.3258
Deficient Smartamine M	39.0	41.5	43.5	44.3	44.9	1.96	<0.0001	0.1061	0.9315

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.

Table 3.13 cont'd. Plasma nonessential AA (NEAA) concentrations (μM) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Gln									
Adequate MetaSmart	230.3	237.4	236.8	239.6	237.8	9.15	0.3609	0.5151	0.8658
Adequate Smartamine M	237.2	245.3	248.0	239.0	237.8	9.15	0.7956	0.1971	0.5051
Deficient MetaSmart	248.4	237.1	249.0	237.7	231.2	9.15	0.0717	0.5391	0.3219
Deficient Smartamine M	258.2	252.5	251.8	246.2	249.1	9.15	0.1913	0.5750	0.8502
Glu									
Adequate MetaSmart	52.7	53.1	53.2	53.7	53.0	2.78	0.7787	0.7272	0.8236
Adequate Smartamine M	60.2	58.4	58.1	58.5	58.7	2.78	0.5055	0.3634	0.7073
Deficient MetaSmart	53.2	52.6	51.7	52.6	52.0	2.78	0.5519	0.7204	0.7869
Deficient Smartamine M	55.6	54.1	56.6	55.2	54.3	2.78	0.7365	0.6146	0.4258
Gly									
Adequate MetaSmart	313.9	327.2	332.3	337.8	347.5	16.46	0.0042	0.8227	0.6470
Adequate Smartamine M	329.8	321.1	331.2	322.5	309.9	16.46	0.1753	0.4219	0.4213
Deficient MetaSmart	388.6	356.7	368.8	359.2	345.0	16.46	0.0018	0.6647	0.0703
Deficient Smartamine M	362.9	372.2	348.4	344.8	345.2	16.46	0.0200	0.9388	0.1654
Hcys									
Adequate MetaSmart	4.0	4.2	4.3	4.1	4.2	0.72	0.5671	0.4766	0.6567
Adequate Smartamine M	4.7	5.3	5.0	4.8	4.8	0.72	0.6456	0.2675	0.1495
Deficient MetaSmart	5.3	5.7	5.6	5.0	5.4	0.72	0.4070	0.6109	0.0660
Deficient Smartamine M	6.5	6.1	6.2	6.3	6.1	0.72	0.3723	0.6540	0.2412

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.

Table 3.13 cont'd. Plasma nonessential AA (NEAA) concentrations (μM) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Orn									
Adequate MetaSmart	47.3	46.2	45.9	48.6	47.5	3.15	0.5570	0.6019	0.3313
Adequate Smartamine M	48.0	49.0	47.0	49.4	45.6	3.15	0.3670	0.3808	0.5224
Deficient MetaSmart	46.6	45.6	45.2	48.2	45.6	3.15	0.8714	0.9733	0.1871
Deficient Smartamine M	45.9	47.4	47.6	45.0	45.8	3.15	0.5716	0.4464	0.3067
Pro									
Adequate MetaSmart	86.5	85.0	87.3	92.1	89.1	3.55	0.0561	0.9506	0.0706
Adequate Smartamine M	84.9	85.7	85.1	83.1	79.8	3.55	0.0596	0.2300	0.9776
Deficient MetaSmart	94.7	90.3	90.1	90.5	88.7	3.55	0.0663	0.4633	0.3180
Deficient Smartamine M	90.4	93.1	92.3	89.1	89.1	3.55	0.3117	0.3067	0.2980
Ser									
Adequate MetaSmart	80.6	82.4	84.3	88.7	86.2	3.52	0.0046	0.4029	0.2608
Adequate Smartamine M	88.0	86.7	88.5	85.9	80.9	3.52	0.0215	0.1236	0.3909
Deficient MetaSmart	98.8	93.7	91.8	93.2	90.8	3.52	0.0074	0.2290	0.2540
Deficient Smartamine M	95.1	95.2	93.1	87.3	86.5	3.52	<0.0001	0.4559	0.2355
Tau									
Adequate MetaSmart	48.4	50.3	52.6	49.4	52.3	2.54	0.1770	0.5365	0.2607
Adequate Smartamine M	54.4	53.4	56.3	53.7	59.4	2.54	0.0556	0.2073	0.4087
Deficient MetaSmart	42.6	44.2	44.8	43.4	44.0	2.54	0.6706	0.4989	0.5470
Deficient Smartamine M	43.7	45.2	46.7	47.3	52.2	2.54	0.0002	0.3117	0.3871
Tyr									
Adequate MetaSmart	45.6	45.7	44.7	46.1	43.4	2.38	0.3715	0.5462	0.4800
Adequate Smartamine M	42.0	46.7	45.6	43.4	41.7	2.38	0.4117	0.0133	0.1801
Deficient MetaSmart	49.0	50.9	48.2	50.5	48.1	2.38	0.6108	0.5115	0.9755
Deficient Smartamine M	45.3	44.7	46.6	43.2	41.0	2.38	0.0254	0.1171	0.7566

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.

Table 3.13 cont'd. Plasma nonessential AA (NEAA) concentrations (μM) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Total NEAA									
Adequate MetaSmart	1399.2	1419.1	1436.9	1467.2	1447.6	37.52	0.0604	0.4644	0.5338
Adequate Smartamine M	1429.6	1438.5	1457.6	1418.8	1391.2	37.52	0.2354	0.1734	0.9890
Deficient MetaSmart	1541.4	1465.2	1490.7	1475.3	1436.7	37.52	0.0103	0.7049	0.1051
Deficient Smartamine M	1489.3	1506.6	1478.6	1454.3	1450.2	37.52	0.0907	0.6662	0.3930
Total SAA[3]									
Adequate MetaSmart	135.0	139.3	143.4	139.0	142.9	4.48	0.0655	0.3583	0.3108
Adequate Smartamine M	141.8	148.5	156.3	155.6	166.5	4.48	<0.0001	0.9978	0.2348
Deficient MetaSmart	110.2	112.2	112.8	113.4	114.4	4.48	0.2605	0.8418	0.8295
Deficient Smartamine M	109.2	117.5	124.4	130.3	137.5	4.48	<0.0001	0.7465	0.7502
Total AA									
Adequate MetaSmart	2462.7	2443.9	2462.5	2501.8	2482.7	60.72	0.4524	0.8962	0.4618
Adequate Smartamine M	2386.1	2445.0	2459.8	2405.6	2348.9	60.72	0.4088	0.0668	0.7634
Deficient MetaSmart	2599.2	2567.1	2517.8	2555.9	2463.3	60.72	0.0311	0.8274	0.3834
Deficient Smartamine M	2513.1	2580.2	2552.9	2500.9	2460.5	60.72	0.1577	0.1212	0.4159

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.
[3] Total SAA = comprised of Met, Cys, Hcys, and Tau.

Table 3.14. Plasma essential AA (EAA) (% total AA) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Arg									
Adequate MetaSmart	3.4	3.4	3.4	3.4	3.4	0.16	0.4441	0.5543	0.8018
Adequate Smartamine M	3.6	3.6	3.5	3.7	3.7	0.16	0.0834	0.1370	0.2633
Deficient MetaSmart	3.6	3.7	3.6	3.6	3.6	0.16	0.5702	0.4156	0.1534
Deficient Smartamine M	3.5	3.5	3.7	3.5	3.6	0.16	0.1836	0.1251	0.4639
His									
Adequate MetaSmart	2.4	2.4	2.5	2.5	2.5	0.11	0.2280	0.2358	0.6800
Adequate Smartamine M	2.4	2.4	2.4	2.5	2.5	0.11	0.4319	0.9071	0.8464
Deficient MetaSmart	2.5	2.5	2.5	2.5	2.5	0.11	0.8162	0.5363	0.9537
Deficient Smartamine M	2.6	2.6	2.5	2.5	2.5	0.11	0.2782	0.1409	0.4539
Ile									
Adequate MetaSmart	5.4	5.1	5.1	5.0	5.0	0.21	0.0438	0.3646	0.6627
Adequate Smartamine M	4.5	4.7	4.5	4.5	4.4	0.21	0.3743	0.5758	0.7576
Deficient MetaSmart	4.8	5.3	4.9	5.1	5.0	0.21	0.5819	0.3428	0.2705
Deficient Smartamine M	4.9	5.1	5.1	5.0	4.8	0.21	0.7215	0.0361	0.9127
Leu									
Adequate MetaSmart	6.5	6.2	6.0	6.0	6.1	0.27	0.0137	0.0397	0.8347
Adequate Smartamine M	5.6	5.8	5.5	5.6	5.5	0.27	0.4854	0.7057	0.5997
Deficient MetaSmart	5.9	6.5	6.0	6.3	6.1	0.27	0.5131	0.2404	0.2464
Deficient Smartamine M	6.0	6.1	6.2	6.1	5.8	0.27	0.2907	0.0547	0.6749
Lys									
Adequate MetaSmart	3.9	3.8	3.9	3.9	3.9	0.13	0.8999	0.9521	0.7059
Adequate Smartamine M	3.9	3.9	3.9	4.0	3.9	0.13	0.9069	0.8197	0.4714
Deficient MetaSmart	3.6	3.8	3.7	3.7	3.7	0.13	0.6657	0.2359	0.5048
Deficient Smartamine M	3.6	3.7	3.9	3.8	3.8	0.13	0.0631	0.0305	0.5696

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.

Table 3.14 cont'd. Plasma essential AA (EAA) (% total AA) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Met									
Adequate MetaSmart	1.6	1.6	1.6	1.6	1.6	0.06	0.6715	0.6094	0.6454
Adequate Smartamine M	1.6	1.8	2.0	2.2	2.4	0.06	<0.0001	0.5466	0.1249
Deficient MetaSmart	0.8	0.9	0.9	0.9	1.0	0.06	0.0010	0.8727	0.6325
Deficient Smartamine M	0.8	1.0	1.1	1.3	1.4	0.06	<0.0001	0.5988	0.4069
Phe									
Adequate MetaSmart	2.0	1.9	1.8	1.8	1.8	0.07	0.0106	0.1945	0.8635
Adequate Smartamine M	1.8	1.9	1.8	1.9	1.9	0.07	0.1467	0.9297	0.9341
Deficient MetaSmart	1.8	1.9	1.8	1.9	1.9	0.07	0.1986	0.6486	0.0873
Deficient Smartamine M	1.8	1.8	1.8	1.8	1.8	0.07	0.7134	0.5480	0.8512
Thr									
Adequate MetaSmart	4.6	4.7	4.6	4.6	4.7	0.18	0.5169	0.9024	0.2317
Adequate Smartamine M	4.6	4.7	4.8	4.7	4.6	0.18	0.4475	0.0365	0.6954
Deficient MetaSmart	5.1	5.0	4.9	5.0	5.0	0.18	0.2384	0.2787	0.7457
Deficient Smartamine M	4.8	4.8	5.0	4.8	4.8	0.18	0.5734	0.1835	0.6117
Trp									
Adequate MetaSmart	1.5	1.5	1.5	1.5	1.5	0.05	0.0400	0.2627	0.2060
Adequate Smartamine M	1.5	1.6	1.5	1.5	1.5	0.05	0.5144	0.3862	0.5230
Deficient MetaSmart	1.5	1.5	1.6	1.6	1.6	0.05	0.4164	0.7926	0.4658
Deficient Smartamine M	1.5	1.5	1.5	1.5	1.5	0.05	0.5822	0.3962	0.1816
Val									
Adequate MetaSmart	12.0	11.4	11.3	11.2	11.3	0.46	0.0390	0.0731	0.6772
Adequate Smartamine M	10.4	10.9	10.6	10.5	10.3	0.46	0.5258	0.1733	0.4496
Deficient MetaSmart	11.0	11.8	10.9	11.6	11.3	0.46	0.6159	0.5000	0.2997
Deficient Smartamine M	11.2	11.5	11.3	11.3	11.0	0.46	0.5321	0.2604	0.8009

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.

Table 3.14 cont'd. Plasma essential AA (EAA) (% total AA) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
EAA									
Adequate MetaSmart	43.2	42.0	41.7	41.3	41.6	1.04	0.0309	0.1416	0.8603
Adequate Smartamine M	40.0	41.2	40.5	41.0	40.7	1.04	0.5223	0.3825	0.5680
Deficient MetaSmart	40.7	42.8	40.7	42.1	41.6	1.04	0.5034	0.4103	0.2021
Deficient Smartamine M	40.6	41.4	41.9	41.7	40.9	1.04	0.5986	0.0555	0.9123
BCAA[3]									
Adequate MetaSmart	23.9	22.6	22.3	22.2	22.4	0.90	0.0245	0.0879	0.7037
Adequate Smartamine M	20.5	21.4	20.6	20.7	20.2	0.90	0.4580	0.3564	0.5449
Deficient MetaSmart	21.7	23.5	21.8	23.0	22.5	0.90	0.5700	0.3609	0.2606
Deficient Smartamine M	22.0	22.6	22.6	22.4	21.6	0.90	0.4788	0.0981	0.9821
Met+Cys									
Adequate MetaSmart	3.4	3.5	3.5	3.4	3.5	0.10	0.1943	0.2016	0.1527
Adequate Smartamine M	3.5	3.7	3.9	4.0	4.3	0.10	<0.0001	0.4400	0.3926
Deficient MetaSmart	2.4	2.4	2.5	2.5	2.6	0.10	0.0001	0.4845	0.8727
Deficient Smartamine M	2.4	2.6	2.8	3.1	3.2	0.10	<0.0001	0.6077	0.3507

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.
[3] BCAA = comprised of values for Leu, Lys, and Valine.

Table 3.15. Plasma nonessential AA (NEAA) (% total AA) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Ala									
Adequate MetaSmart	12.2	12.0	12.2	12.5	11.7	0.48	0.6373	0.2330	0.0880
Adequate Smartamine M	12.3	11.9	12.2	12.0	12.1	0.48	0.8181	0.5362	0.6805
Deficient MetaSmart	12.0	11.3	11.7	11.5	11.9	0.48	0.9677	0.0979	0.4672
Deficient Smartamine M	11.5	11.6	11.3	11.7	11.4	0.48	0.9875	0.9894	0.7979
Asn									
Adequate MetaSmart	2.2	2.3	2.3	2.4	2.3	0.06	0.0134	0.0346	0.3366
Adequate Smartamine M	2.2	2.2	2.2	2.2	2.1	0.06	0.0662	0.0469	0.9364
Deficient MetaSmart	2.4	2.3	2.4	2.4	2.3	0.06	0.4767	0.7947	0.2821
Deficient Smartamine M	2.3	2.3	2.3	2.3	2.3	0.06	0.4418	0.2492	0.3131
Asp									
Adequate MetaSmart	0.1	0.1	0.1	0.1	0.1	0.01	0.1682	0.3920	0.4896
Adequate Smartamine M	0.1	0.1	0.1	0.1	0.1	0.01	0.3908	0.8538	0.7196
Deficient MetaSmart	0.1	0.1	0.1	0.1	0.1	0.01	0.9265	0.8152	0.4612
Deficient Smartamine M	0.1	0.1	0.1	0.1	0.1	0.01	1.0000	0.7553	0.1682
Cit									
Adequate MetaSmart	3.7	3.7	3.6	3.5	3.6	0.20	0.4073	0.8531	0.2846
Adequate Smartamine M	3.7	3.8	3.6	3.8	3.9	0.20	0.4188	0.1625	0.2071
Deficient MetaSmart	3.7	3.8	3.9	3.8	3.8	0.20	0.5444	0.2337	0.7101
Deficient Smartamine M	4.0	3.7	3.9	3.8	4.0	0.20	0.7483	0.0305	0.8326
Cys									
Adequate MetaSmart	1.8	1.9	1.9	1.8	1.9	0.07	0.1146	0.1542	0.0720
Adequate Smartamine M	1.9	1.8	1.9	1.9	1.9	0.07	0.1922	0.5897	0.6294
Deficient MetaSmart	1.6	1.6	1.6	1.6	1.7	0.07	0.0176	0.3202	0.8054
Deficient Smartamine M	1.6	1.6	1.7	1.8	1.8	0.07	<0.0001	0.8139	0.5704

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.

Table 3.15 cont'd. Plasma nonessential AA (NEAA) (% total AA) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Gln									
Adequate MetaSmart	9.4	9.7	9.6	9.6	9.6	0.38	0.6262	0.4904	0.4883
Adequate Smartamine M	10.0	10.0	10.3	10.0	10.2	0.38	0.6281	0.8653	0.6725
Deficient MetaSmart	9.6	9.3	10.0	9.3	9.4	0.38	0.6994	0.4911	0.7692
Deficient Smartamine M	10.3	9.8	9.9	9.9	10.2	0.38	0.7492	0.0887	0.7210
Glu									
Adequate MetaSmart	2.2	2.2	2.2	2.2	2.1	0.12	0.6776	0.6622	0.7445
Adequate Smartamine M	2.6	2.4	2.4	2.5	2.5	0.12	0.9322	0.0193	0.4732
Deficient MetaSmart	2.1	2.1	2.1	2.1	2.1	0.12	0.4933	0.6970	0.9061
Deficient Smartamine M	2.2	2.1	2.2	2.2	2.2	0.12	0.6410	0.6316	0.2156
Gly									
Adequate MetaSmart	12.7	13.4	13.5	13.5	14.0	0.61	0.0022	0.7174	0.2505
Adequate Smartamine M	13.8	13.1	13.6	13.4	13.2	0.61	0.2446	0.6782	0.2335
Deficient MetaSmart	15.0	13.9	14.7	14.2	14.0	0.61	0.0621	0.5614	0.1236
Deficient Smartamine M	14.5	14.5	13.7	13.8	14.0	0.61	0.0758	0.1787	0.3311
Hcys									
Adequate MetaSmart	0.2	0.2	0.2	0.2	0.2	0.03	0.7246	0.3724	0.5645
Adequate Smartamine M	0.2	0.2	0.2	0.2	0.2	0.03	0.9645	0.4546	0.1660
Deficient MetaSmart	0.2	0.2	0.2	0.2	0.2	0.03	0.8981	0.6262	0.0309
Deficient Smartamine M	0.3	0.2	0.3	0.3	0.3	0.03	0.6772	0.4020	0.2129

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.

Table 3.15 cont'd. Plasma nonessential AA (NEAA) (% total AA) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Orn									
Adequate MetaSmart	1.9	1.9	1.9	1.9	1.9	0.11	0.7196	0.5276	0.4964
Adequate Smartamine M	2.0	2.0	1.9	2.1	1.9	0.11	0.4114	0.9232	0.2446
Deficient MetaSmart	1.8	1.8	1.8	1.9	1.8	0.11	0.1344	0.8348	0.2129
Deficient Smartamine M	1.8	1.8	1.9	1.8	1.9	0.11	0.8575	0.8102	0.4917
Pro									
Adequate MetaSmart	3.5	3.5	3.5	3.7	3.6	0.10	0.0140	0.7888	0.0210
Adequate Smartamine M	3.5	3.5	3.4	3.4	3.4	0.10	0.0148	0.8448	0.7120
Deficient MetaSmart	3.6	3.5	3.6	3.5	3.6	0.10	0.5781	0.1277	0.5173
Deficient Smartamine M	3.6	3.6	3.6	3.6	3.6	0.10	0.8991	0.7299	0.4225
Ser									
Adequate MetaSmart	3.3	3.4	3.4	3.5	3.5	0.12	0.1391	0.3068	0.4338
Adequate Smartamine M	3.7	3.6	3.6	3.6	3.5	0.12	0.0067	0.7647	0.0799
Deficient MetaSmart	3.8	3.7	3.7	3.7	3.7	0.12	0.1529	0.0528	0.4263
Deficient Smartamine M	3.8	3.7	3.7	3.5	3.5	0.12	<0.0001	0.5428	0.3701
Tau									
Adequate MetaSmart	2.0	2.1	2.2	2.0	2.1	0.10	0.3108	0.5393	0.0841
Adequate Smartamine M	2.3	2.2	2.3	2.2	2.5	0.10	0.0039	0.0136	0.4047
Deficient MetaSmart	1.6	1.7	1.8	1.7	1.8	0.10	0.1404	0.5013	0.3902
Deficient Smartamine M	1.7	1.8	1.8	1.9	2.1	0.10	<0.0001	0.0442	0.5749
Tyr									
Adequate MetaSmart	1.9	1.9	1.8	1.9	1.8	0.09	0.0613	0.3507	0.6974
Adequate Smartamine M	1.8	1.9	1.8	1.8	1.8	0.09	0.5598	0.0428	0.1099
Deficient MetaSmart	1.9	2.0	1.9	2.0	2.0	0.09	0.3852	0.5195	0.5320
Deficient Smartamine M	1.8	1.7	1.8	1.7	1.7	0.09	0.0396	0.2686	0.3186

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.

Table 3.15 cont'd. Plasma nonessential AA (NEAA) (% total AA) of Holstein cows fed Met-adequate or Met-deficient diets supplemented with incremental amounts of Smartamine M or MetaSmart[1].

	Methionine equivalents (g/d per 24 kg DMI)						*P* =		
Item	0	3	6	9	12	SE[2]	L	Q	C
Total NEAA									
Adequate MetaSmart	56.8	58.0	58.4	58.7	58.4	1.04	0.0309	0.1416	0.8603
Adequate Smartamine M	60.0	58.8	59.5	59.0	59.3	1.04	0.5223	0.3825	0.5680
Deficient MetaSmart	59.3	57.2	59.4	57.9	58.4	1.04	0.5034	0.4103	0.2021
Deficient Smartamine M	59.4	58.6	58.1	58.3	59.1	1.04	0.5986	0.0555	0.9123
Total SAA[3]									
Adequate MetaSmart	5.5	5.7	5.8	5.6	5.8	0.13	0.1141	0.1901	0.0253
Adequate Smartamine M	5.9	6.1	6.4	6.5	7.1	0.13	<0.0001	0.0254	0.1974
Deficient MetaSmart	4.2	4.4	4.5	4.4	4.6	0.13	0.0006	0.8914	0.3058
Deficient Smartamine M	4.3	4.6	4.9	5.2	5.6	0.13	<0.0001	0.1890	0.7650

[1] Smartamine M was included in the Met adequate diet in an amount sufficient to generate a 3.0:1 Lys/Met ratio in MP.
[2] Largest standard error of treatment means is shown.
[3] Total SAA = comprised of Met, Cys, Hcys, and Tau.

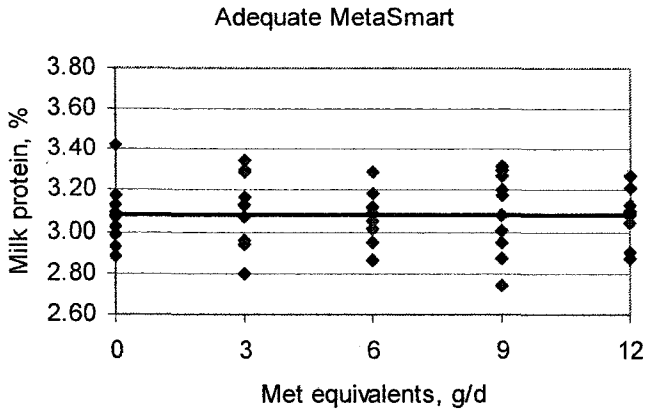
Table 3.16. Met "bioavailability" of Met-adequate and Met-deficient MetaSmart relative to that of Met-adequate and Met-deficient Smartamine M, respectively, using changes in milk protein concentration (Figure 3.1), plasma Met concentration (μM) (Figure 3.2), plasma Met (% total AA) (Figure 3.3), plasma Met+Cys concentration (μM) (Figure 3.4), plasma Met+Cys (% total AA) (Figure 3.5), plasma total sulfur AA concentration (μM) (Figure 3.6), and plasma total sulfur AA (% total AA) (Figure 3.7) as the response criteria.

Item	Adequate Smartamine M[1]	Adequate MetaSmart[2]	Deficient Smartamine M[2]	Deficient MetaSmart[2]
Milk protein, %				
Slope	0.0007[4]	-0.0003[4]	0.0063[3]	0.0083[3]
95% confidence interval	-0.0101 – 0.0116	-0.0100 – 0.0095	-0.0033 – 0.0159	-0.0013 – 0.0179
SE	0.006	0.005	0.006	0.008
(MetaSmart/Smartamine M)[5]	-	-	-	131.7 ± 152.3
Plasma Met, μM				
Slope	1.5311[3]	0.1052[4]	1.2100[3]	0.2273[4]
95% confidence interval	1.5221 – 1.5401	0.0955 – 0.1149	1.2053 – 1.2148	0.2182 – 0.2365
SE	0.345	0.207	0.098	0.092
(MetaSmart/Smartamine M)[5]	-	-	-	-
Plasma Met, % total AA				
Slope	0.0666[3]	0.0016[4]	0.0514[3]	0.0124[3]
95% confidence interval	0.0587 – 0.0744	-0.0081 – 0.0113	0.0477 – 0.0550	0.0041 – 0.0207
SE	0.011	0.006	0.003	0.003
(MetaSmart/Smartamine M)[5]	-	-	-	24.1 ± 16.2
Plasma Met+Cys, μM				
Slope	1.5637[3]	0.2786[4]	1.6970[3]	0.2636[4]
95% confidence interval	1.5541 – 1.5734	0.2690 – 0.2882	1.6898 – 1.7042	0.0254 – 0.2732
SE	0.469	0.298	0.272	0.279
(MetaSmart/Smartamine M)[5]	-	-	-	-

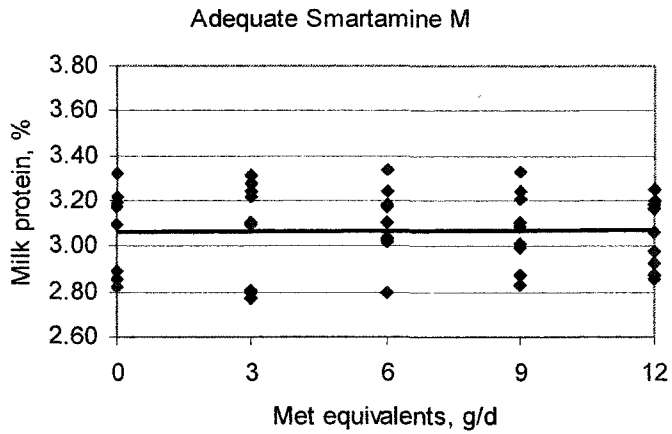
Table 3.16 cont'd

Plasma Met+Cys, % total AA				
Slope	0.0710[3]	0.0065[4]	0.0745[3]	0.0199[3]
95% confidence interval	0.0626 – 0.0795	-0.0032 – 0.0162	0.0688 – 0.0802	0.0107 – 0.0292
SE	0.013	0.009	0.008	0.009
(MetaSmart/Smartamine M)[5]	-	-	-	26.7 ± 12.4
Plasma total sulfur AA, μM				
Slope	1.8855[3]	0.5175[4]	2.3112[3]	0.3149[4]
95% confidence interval	1.8754 – 1.8956	0.5079 – 0.5271	2.3033 – 2.3192	0.3052 – 0.3246
SE	0.735	0.482	0.479	0.503
(MetaSmart/Smartamine M)[5]	-	-	-	-
Plasma total sulfur AA, % total AA				
Slope	0.0903[3]	0.0131[4]	0.1051[3]	0.0289[3]
95% confidence interval	0.0810 – 0.0995	0.0034 – 0.0228	0.0987 – 0.1115	0.0196 – 0.0383
SE	0.022	0.016	0.013	0.015
(MetaSmart/Smartamine M)[5]	-	-	-	27.5 ± 8.9

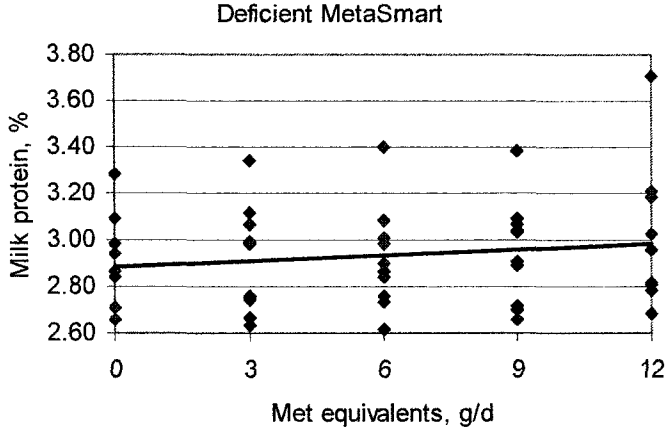
[1] Results based on n = 45 observations (9 observations per Met supplementation level).
[2] Results based on n = 50 observations (10 observations per Met supplementation level).
[3] Linear contrast of treatment means significant at $P < 0.05$ for incremental amounts Adequate Smartamine M, Adequate MetaSmart, Deficient Smartamine M or Deficient MetaSmart.
[4] Linear contrast of treatment means non-significant at $P < 0.05$ for incremental amounts Adequate Smartamine M, Adequate MetaSmart, Deficient Smartamine M or Deficient MetaSmart.
[5] Calculated as slope of Adequate or Deficient MetaSmart divided by the slope of Adequate or Deficient Smartamine M, respectively, and is based on an assumed bioavailability of 80% for Smartamine M



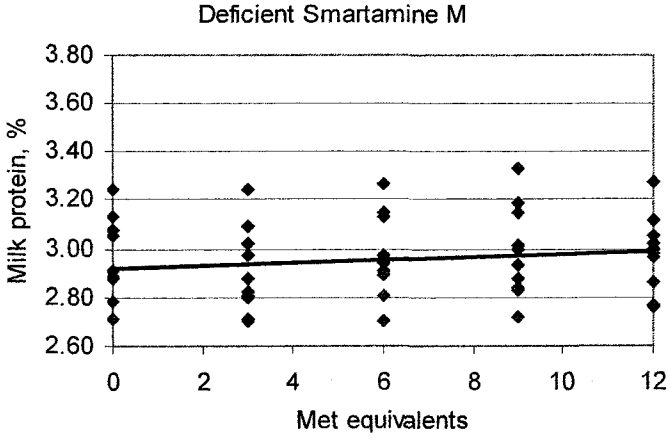
Y = 3.0872 – 0.0003x; R^2 = -0.0208; SE = 0.1529; n = 50.



Y = 3.0678 + 0.0007x; R^2 = -0.0229; SE = 0.1731; n = 45.

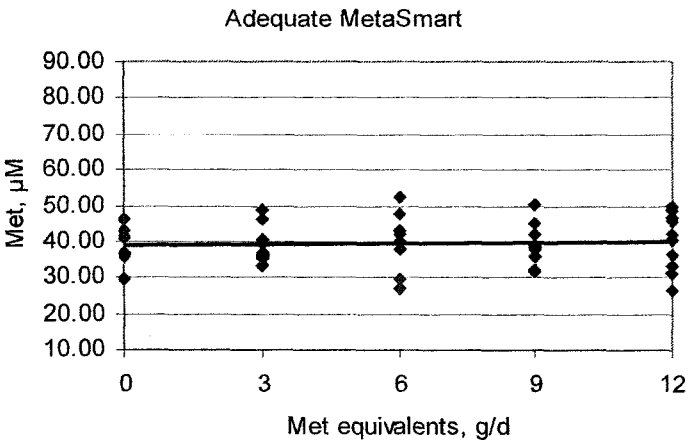


Y = 2.8846 + 0.0083x; R^2 = 0.0030; SE = 0.2332; n = 50.

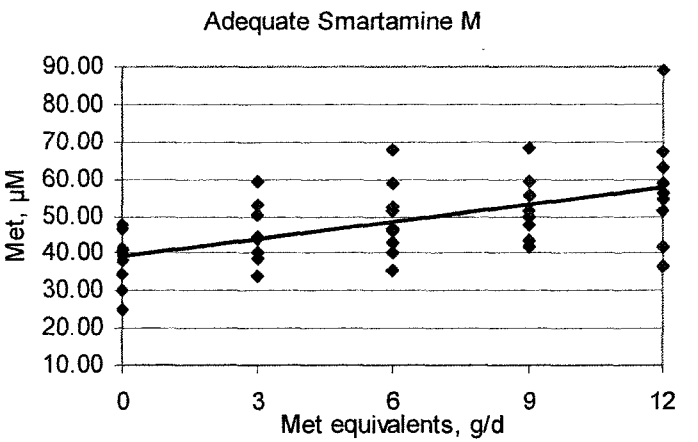


Y = 2.919 + 0.0063x; R^2 = 0.0036; SE = 0.1751; n = 50.

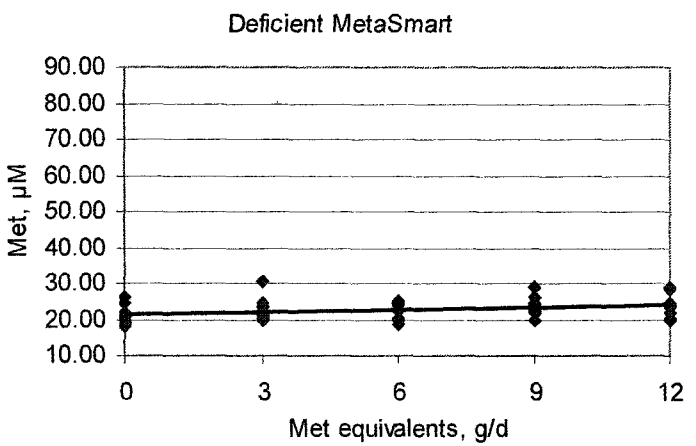
Figure 3.1. Effects of feeding incremental amounts of MetaSmart or Smartamine M to cows receiving Met-adequate or Met-deficient diets on milk protein concentrations. The equation for the regression line of each plot is provided below the respective plot.



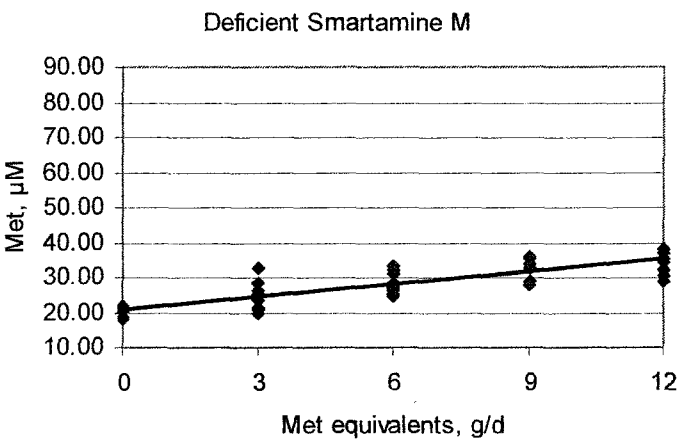
$Y = 38.932 + 0.1052x$; $R^2 = -0.0154$; SE = 6.1982; n = 50.



$Y = 39.076 + 1.5311x$; $R^2 = 0.2980$; SE = 9.8229; n = 45.

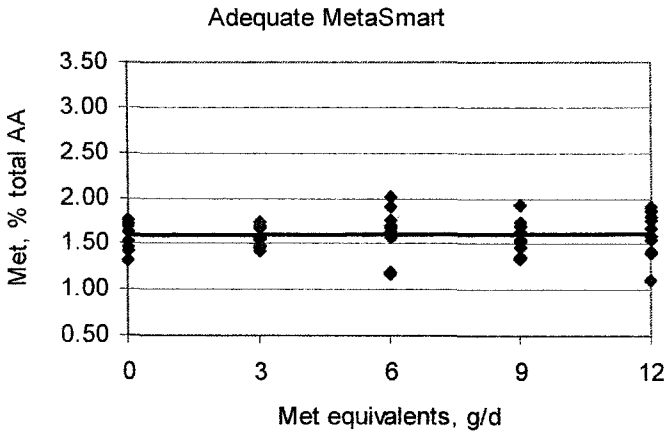


$Y = 21.235 + 0.2273x$; $R^2 = 0.0944$; SE = 2.7597; n = 50.

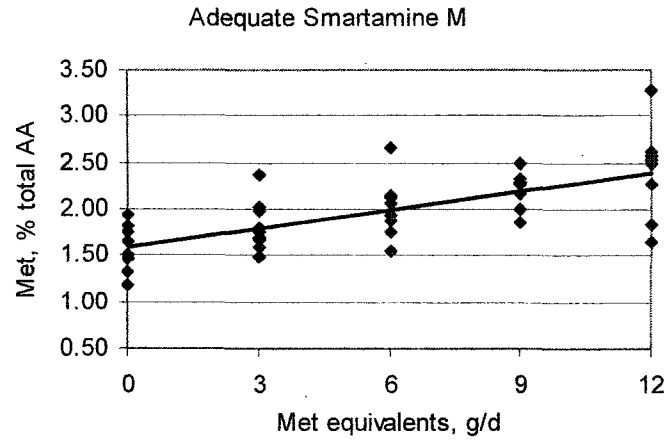


$Y = 20.637 + 1.21x$; $R^2 = 0.7563$; SE = 2.9339; n = 50.

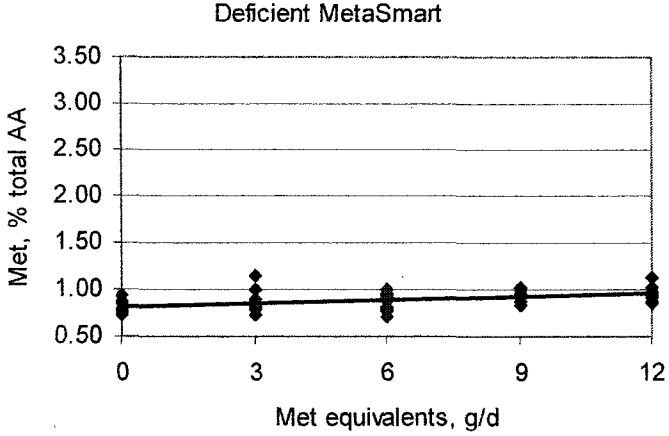
Figure 3.2. Effect of feeding incremental amounts of MetaSmart or Smartamine M to cows receiving Met-adequate or Met-deficient diets on plasma Met concentrations (µM). The equation for the regression line of each plot is provided below the respective plot.



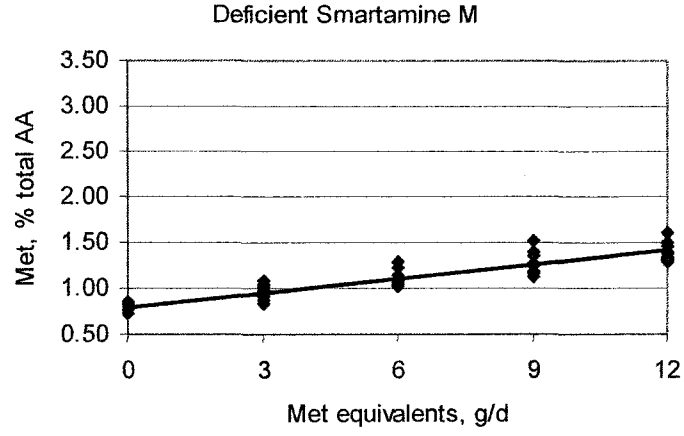
$Y = 1.5882 + 0.0016x$; $R^2 = -0.0196$; SE = 0.1936; n = 50.



$Y = 1.5929 + 0.0666x$; $R^2 = 0.4590$; SE = 0.306; n = 45.

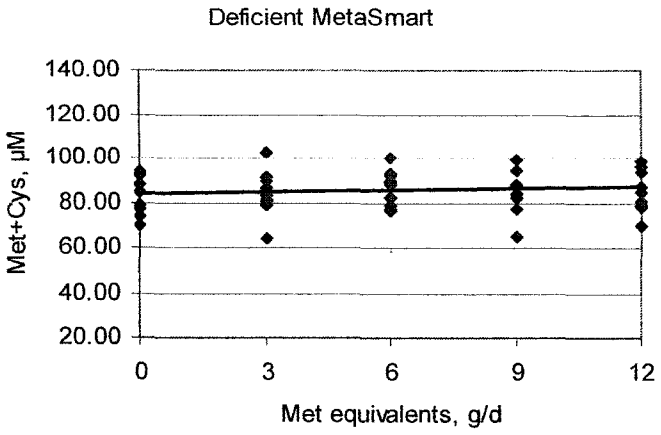


$Y = 0.8164 + 0.0124x$; $R^2 = 0.2594$; SE = 0.0871; n = 50.

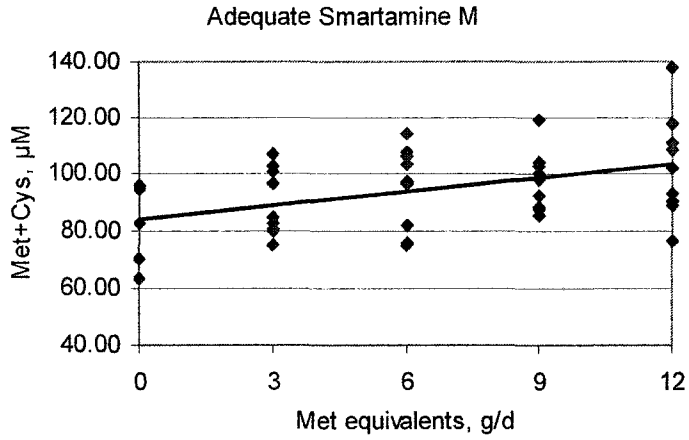


$Y = 0.8006 + 0.0514x$; $R^2 = 0.8565$; SE = 0.09; n = 50.

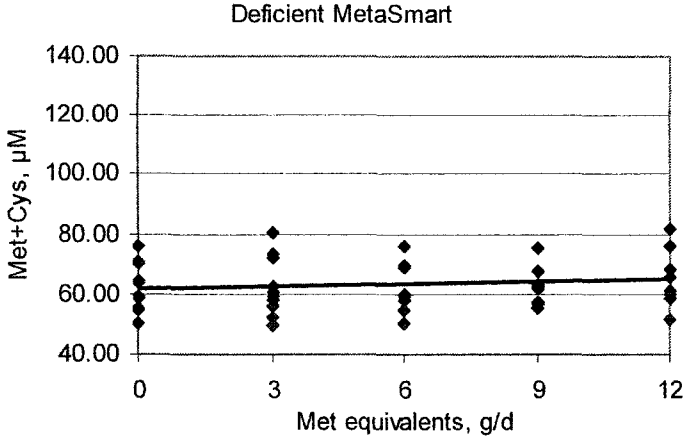
Figure 3.3. Effect of feeding incremental amounts of MetaSmart or Smartamine M to cows receiving Met-adequate or Met-deficient diets on plasma Met concentrations (% total AA). The equation for the regression line of each plot is provided below the respective plot.



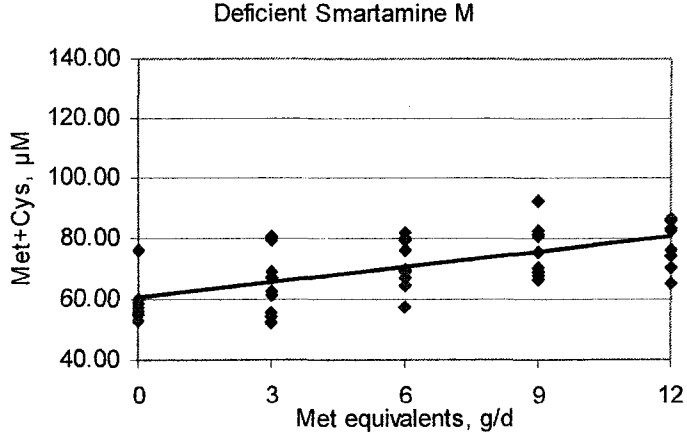
Y = 83.479 + 0.2786x; R^2 = -0.0026; SE = 8.9531; n = 50.



Y = 84.456 + 1.5637x; R^2 = 0.1870; SE = 13.346; n = 45.

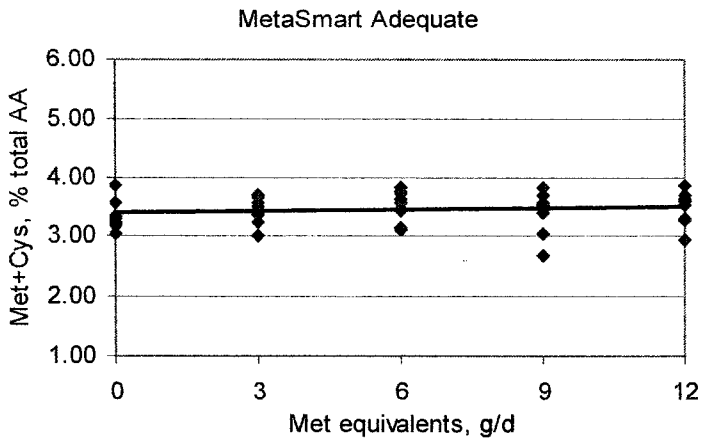


Y = 61.822 + 0.2636x; R^2 = -0.0022; SE = 8.3764; n = 50.

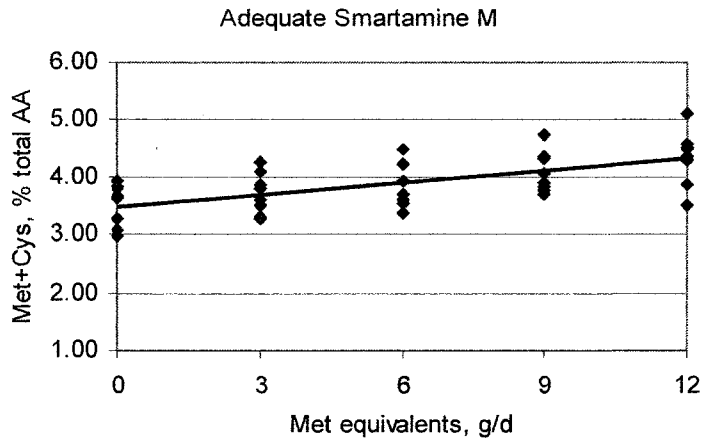


Y = 60.361 + 1.697x; R^2 = 0.4365; SE = 8.1574; n = 50.

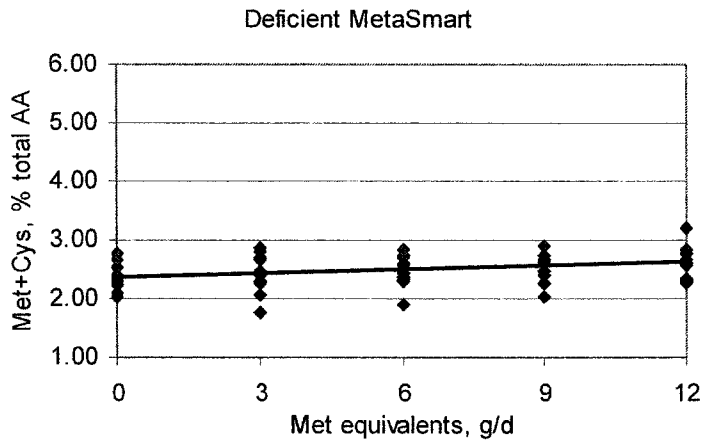
Figure 3.4. Effect of feeding incremental amounts of MetaSmart or Smartamine M to cows receiving Met-adequate or Met-deficient diets on plasma Met+Cys concentrations (µM). The equation for the regression line of each plot is provided below the respective plot.



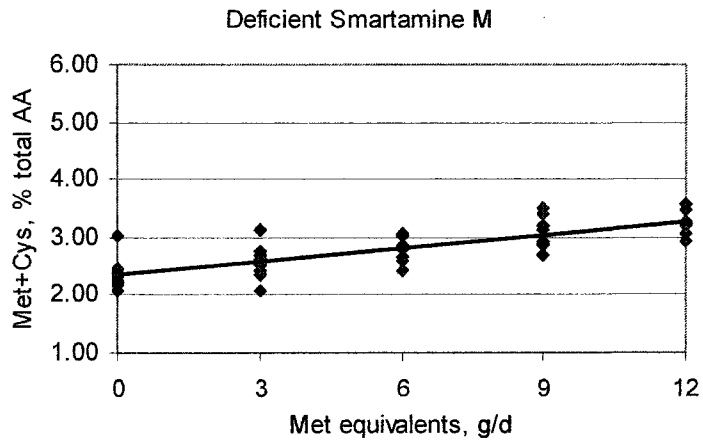
$Y = 3.406 + 0.0065x$; $R^2 = -0.0089$; SE = 0.2592; n = 50.



$Y = 3.458 + 0.071x$; $R^2 = 0.3818$; SE = 0.3809; n = 45.

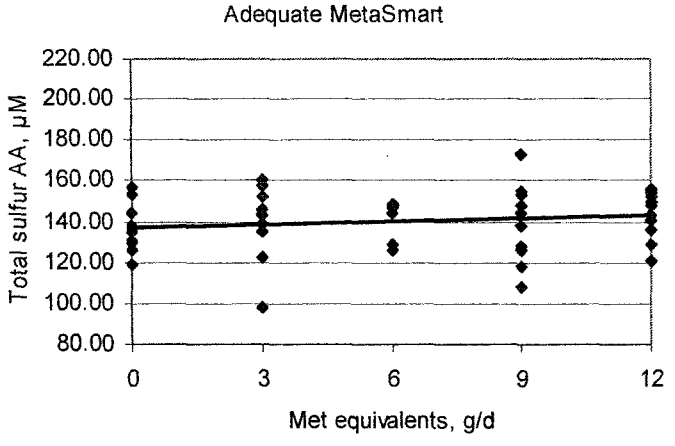


$Y = 2.3778 + 0.0199x$; $R^2 = 0.0726$; SE = 0.2719; n = 50.

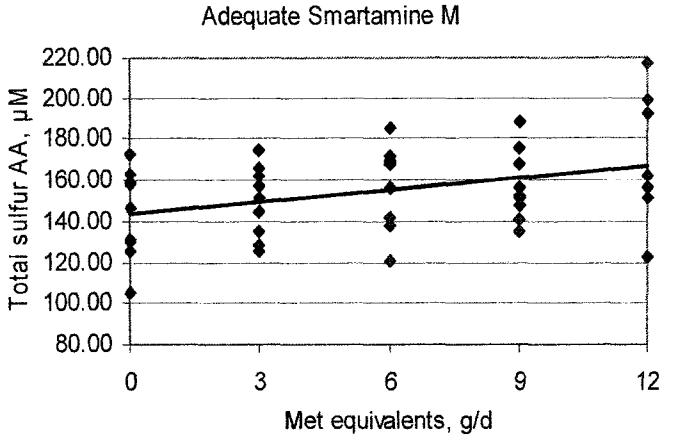


$Y = 2.3536 + 0.0745x$; $R^2 = 0.6464$; SE = 0.2348; n = 50.

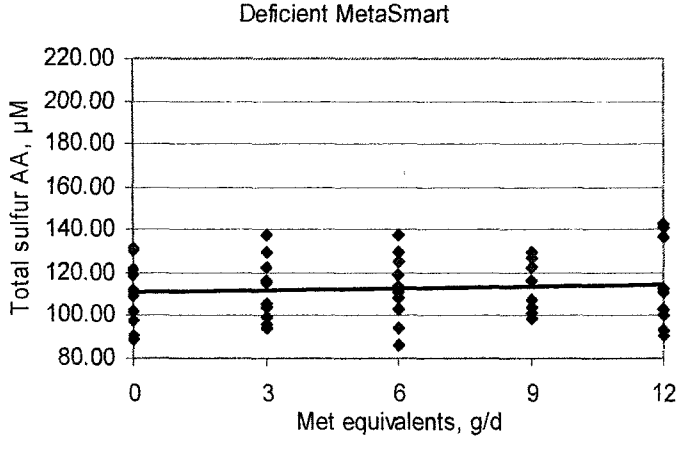
Figure 3.5. Effect of feeding incremental amounts of MetaSmart or Smartamine M to cows receiving Met-adequate or Met-deficient diets on plasma Met+Cys concentrations (% of total AA). The equation for the regression line of each plot is provided below the respective plot.



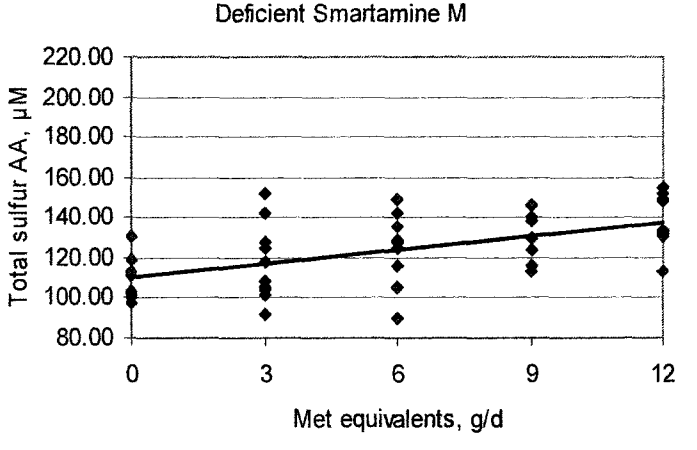
Y = 136.81 + 0.5175x; R^2 = 0.0031; SE = 14.455; n = 50.



Y = 143.85 + 1.8855x; R^2 = 0.1126; SE = 20.914; n = 45.

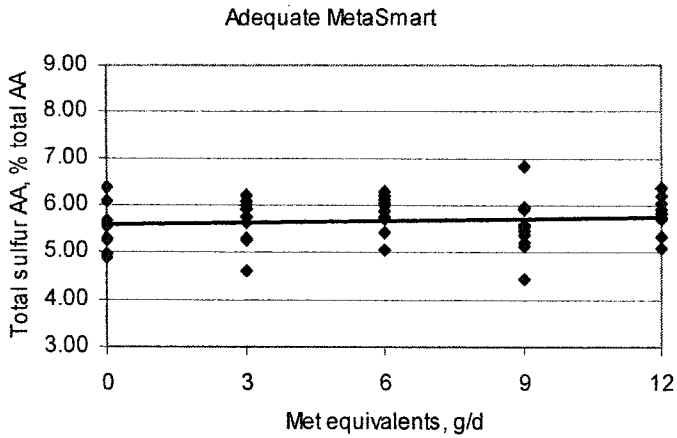


Y = 110.69 + 0.3149x; R^2 = -0.0126; SE = 15.077; n = 50.

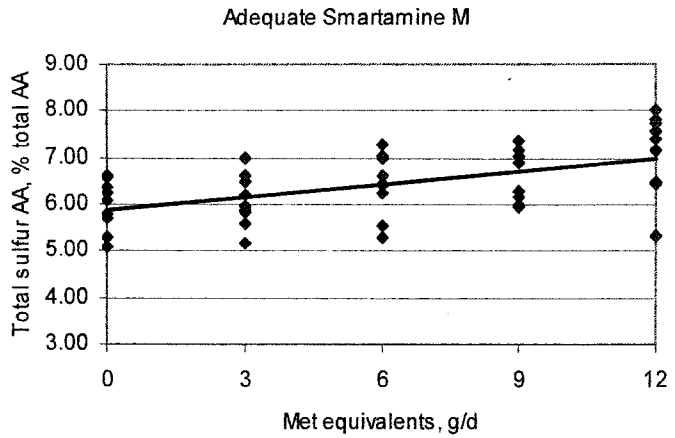


Y = 109.93 + 2.3112x; R^2 = 0.3126; SE = 14.369; n = 50.

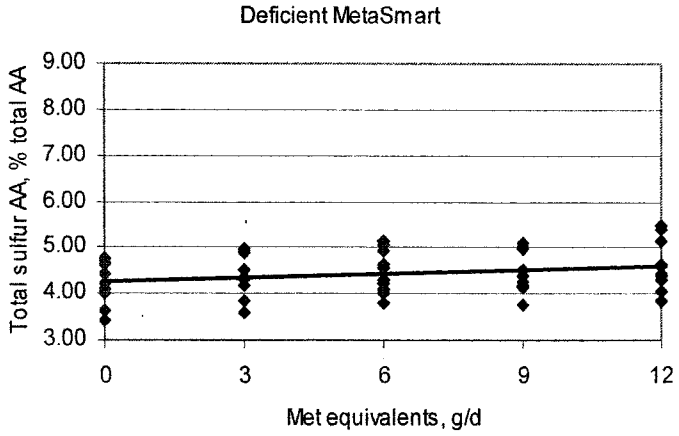
Figure 3.6. Effect of feeding incremental amounts of MetaSmart or Smartamine M to cows receiving Met-adequate or Met-deficient diets on plasma total sulfur AA concentrations (µM). The equation for the regression line of each plot is provided below the respective plot.



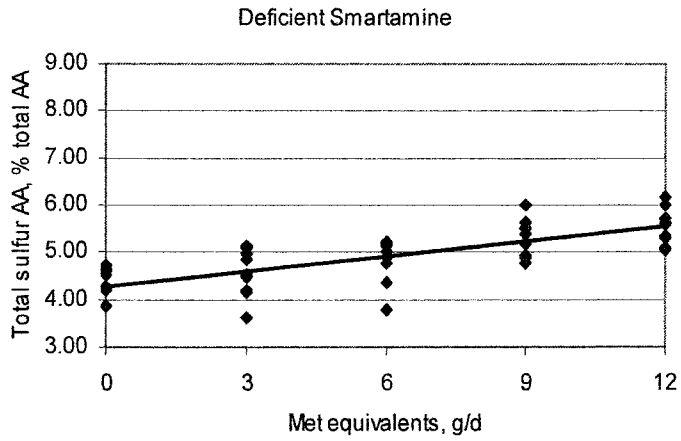
Y = 5.587 + 0.0131x; R^2 = -0.0068; SE = 0.48; n = 50.



Y = 5.8862 + 0.0903x; R^2 = 0.2593; SE = 0.6343; n = 45.



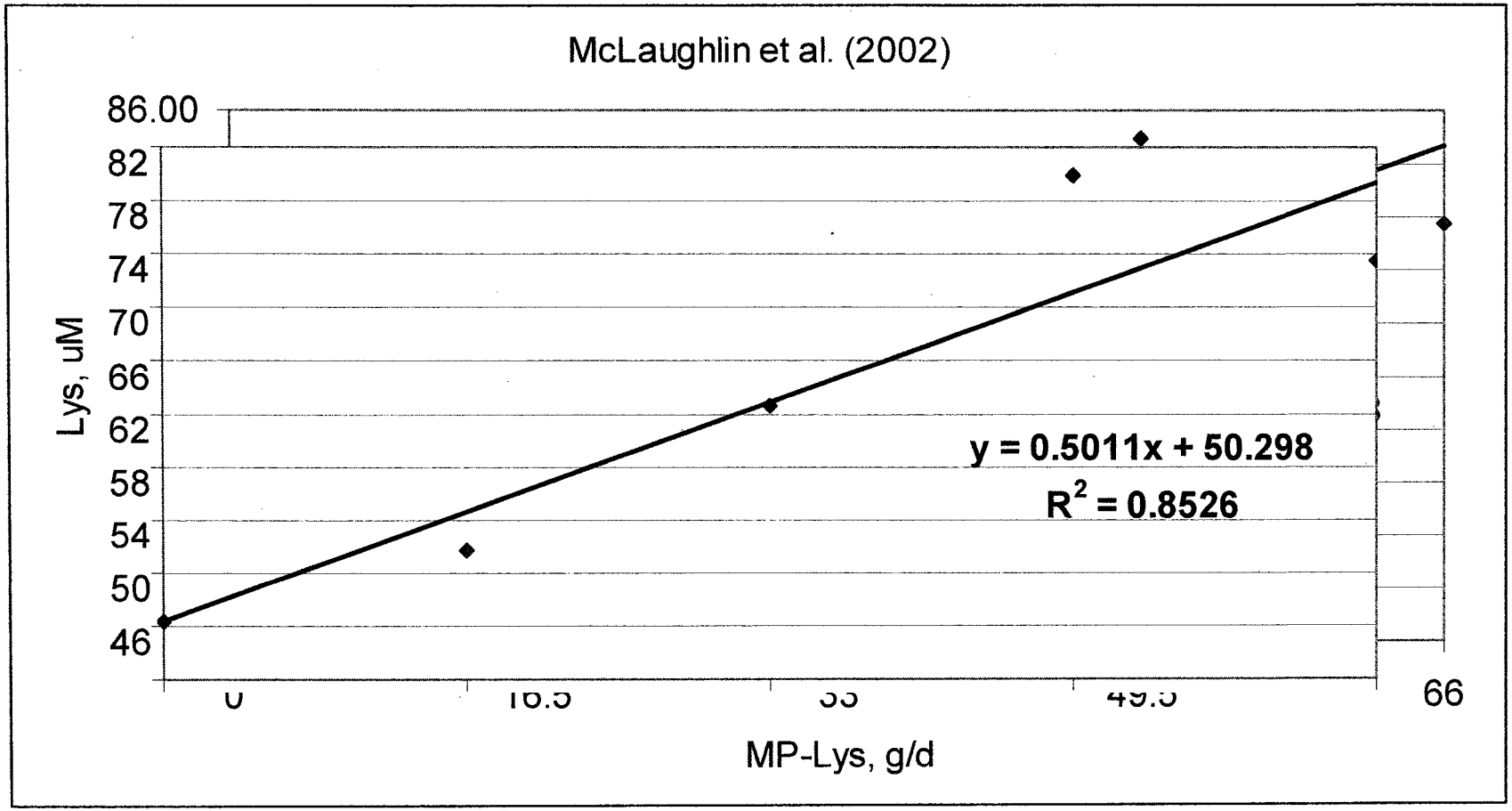
Y = 4.2552 + 0.0289x; R^2 = 0.0488; SE = 0.463; n = 50.



Y = 4.2552 + 0.0289x; R^2 = 0.0488; SE = 0.463; n = 50.

Figure 3.7. Effect of feeding incremental amounts of MetaSmart or Smartamine M to cows receiving Met-adequate or Met-deficient diets on plasma total sulfur AA concentrations (% total AA). The equation for the regression line of each plot is provided below the respective plot.

Figure 3.8. Plot of plasma Lys concentrations (μM) regressed on incremental levels of infused LysHCl (adapted from McLaughlin et al.; 2002).



CHAPTER IV

A COMPARISON OF THE RELATIONSHIPS BETWEEN PREDICTED SUPPLIES OF METABOLIZABLE PROTEIN (MP), MP-METHIONINE, AND MP-LYSINE AS PREDICTED BY NRC (2001) AND ACTUAL YIELD OF MILK AND MILK PROTEIN

ABSTRACT

In the year 2002, dairy producers and nutritionists observed a severe decline in the price of milk. As a result, interest in reducing the cost of rations peaked and the addition of expensive protein supplements in dairy rations such as, blood meal and supplemental Met sources was questioned. In response to this situation, Schwab et al. (2003) attempted to generate prediction equations for determining expected responses in milk and milk protein yield with different levels of metabolizable protein (**MP**), MP-Met, and MP-Lys. The goals of this study were to extend the preliminary work of Schwab et al. (2003; 2004) of predicting milk and milk protein yield from metabolizable protein, Lys and Met and to determine whether using the first limiting amino acid for milk protein production is more accurate than using metabolizable protein to predict milk and milk protein yield. Predicted supplies of MP-Met and MP-Lys than MP, results from 464 diets published in the *Journal of Dairy Science* were entered into the NRC (2001) model and results from the Summary and Duodenal Amino Acid Supply Reports were used to generate plots of

measured milk and milk protein yield vs. predicted supplies of MP, MP-Met, and MP-Lys (g/d). Several restrictions on the dataset for generating plots of measured milk and milk protein yield vs. predicted supplies of MP, MP-Met, and MP-Lys (g/d) were imposed based on NRC (2001) dietary predictions. They were: 1) MP-balance ranged from -300 to 100 g/d, 2) RDP balance had to be greater than -200 g/d, 3) Lys as a percent of MP had to be less than 7.2% and Met as a percent of MP had to be less than 2.4%, 4) for plots based on MP-Met flows, diets had to have a Lys to Met ratio of greater than or equal to 3.0:1, and 5) for plots based on MP-Lys flows, diets had to have a Lys to Met ratio of less than or equal to 3.0:1. The following regression equations describe the relationship between measured milk yield and MP, MP-Met and MP-Lys supplies, respectively: MP (n = 153): $y = -1.9003 + 0.0164x$, $R^2 = 0.81$, SE = 3.04; MP-Met (n = 141): $y = 2.3205 + 0.7551x$, $R^2 = 0.69$, SE = 3.82; MP-Lys (n = 16): $y = 3.3641 + 0.2146x$, $R^2 = 0.95$, SE = 1.86 and between milk protein yield and MP, MP-Met and MP-Lys, respectively: MP (n =153): $y = -137.61 + 0.5183x$, $R^2 = 0.89$, SE = 69.09; MP-Met (n = 141): $y = -12.299 + 24.027x$, $R^2 = 0.77$, SE = 98.87; MP-Lys (n = 16): $y = 91.461 + 6.564x$, $R^2 = 0.94$, SE = 62.78, respectively. This study demonstrated that prediction equations based on NRC (2001) predicted flows of MP-Lys may be more accurate at predicting milk and milk protein yield than MP or MP-Met flows when Lys is either the first limiting AA or co-limiting with Met. In addition, predicting milk protein yield from flows of MP, MP-Met and MP-Lys is more accurate than predicting milk yield.

(**Key words:** methionine, lysine, metabolizable protein)

Abbreviation key: EAA = essential amino acid, **MP** = metabolizable protein.

INTRODUCTION

In the year 2002, dairy producers and nutritionists observed a severe decline in the price of milk. As a result, interest in reducing the cost of rations peaked and the addition of expensive protein supplements in dairy rations such as, blood meal, protected soy products, and protected Met products was questioned. In response to this situation, Schwab et al. (2003) attempted to generate prediction equations for determining expected responses in milk and milk protein yield with different levels of MP, MP-Met, and MP-Lys. The goals were to determine whether prediction equations could be developed to predict milk and milk protein yield from NRC (2001) predicted flows of MP, MP-Met, and MP-Lys and to determine which parameter (i.e., MP, MP-Met, or MP-Lys) was the best predictor of milk and milk protein yield. If equations could be developed, nutritionists could utilize them to determine a rough estimation of the expected economic return from increased milk and milk protein yield in response to adding additional amounts of MP, MP-Met or MP-Lys to their rations. This would help them to determine whether it was more beneficial to continue to keep expensive protein supplements such as, blood meal, protected soy products, and protected Met products in their rations, reduce their inclusion rates, or remove them completely and replace them with lower cost, lower quality feeds.

The NRC (2001) predicts passage of MP to the small intestine and the content of essential AA (**EAA**) in MP. From these values, flows of MP-AA are calculated. The model predicts milk yield from predicted flows of MP; however, it does not predict milk protein yield. Schwab et al. (2003) entered the diet composition and production data of

published studies into NRC (2001) and then used the results to generate prediction equations. In a subsequent effort, Schwab et al. (2004) utilized the same dataset but made further refinements. The prediction equations developed by Schwab et al. (2004) were derived by regressing milk or milk protein yield on NRC (2001) predicted flows of MP, MP-Lys and MP-Met. Their results indicated that flows of MP-Lys were the most accurate at predicting milk and milk protein yield followed by MP-Met and MP; however, the dataset was restricted to include only 28 of the original 321 diets when using flows of MP-Lys as a predictor. Regardless, the study was a first step in determining prediction equations for milk and milk protein yield in response to changes in supplies of MP, MP-Lys and MP-Met.

Doepel et al. (2004) conducted a similar but more technical approach using a more refined dataset than that used by Schwab et al. (2004). The first goal of the study was to generate prediction equations for milk protein yield and changes in milk protein yield in response to changes in intestinal AA supply. The second goal was to test the hypothesis that the efficiency of conversion of digestible AA into milk protein is not constant as is assumed by current models such as NRC (2001) and CNCPS (2000). The authors were unable to detect a significant relationship between the extent and direction of the change in milk protein yield and changes in total AA supply. As a result, regression equations predicting changes in milk protein yield in response to supplementary AA were not developed. The authors demonstrated that the efficiency of conversion of digestible AA into milk protein is variable and not constant as is assumed by NRC (2001) and CNCPS (2000).

The goals of this study were to extend the preliminary work of Schwab et al. (2003) and Schwab et al. (2004) of predicting milk and milk protein yield from MP, MP-Met, and MP-Lys and to determine whether using the first limiting/co-limiting amino acid for milk protein production is more accurate than using metabolizable protein to predict milk and milk protein yield. It must be noted that the prediction equations developed in this study were not intended to be incorporated into models such as the NRC (2001) and only represent a more refined approach of the work conducted by Schwab et al. (2003) and Schwab et al. (2004). This study represents only a preliminary attempt at utilizing published research studies to determine the association between predicted MP, MP-Met, and MP-Lys flows and milk and milk protein yields.

MATERIALS AND METHODS

Source of Data and Criteria Used in Analyses

Data used in the analyses of this study were derived from studies published only in the *Journal of Dairy Science*. A total of 464 experimental diets from 105 experiments in 83 published studies comprised the dataset. Several studies consisted of multiple experiments, however, each diet from each respective experiment was considered to be a separate data point.

The development of the prediction equations was based on entering the results of published studies into the NRC (2001) model and using the predicted dietary flows of MP, Met and Lys. As a result, several restrictions were imposed on the dataset prior to entry into the NRC (2001) model in an effort to reduce grossly inaccurate predictions by

the NRC (2001) model in relation to requirements. Restrictions included differences related to breed of cow, forage types and studies in which the physiological behavior of supplementary AA products being tested were not thoroughly understood or documented. The restrictions imposed were: 1) only studies in which Holstein cows were used were included, 2) only studies in which chemical compositions of all forages used in the study were provided; however, if the nutrient compositions for feeds other than forages were provided, then these values were used, otherwise NRC (2001) default values were used and 3) studies in which protected AA products were fed were included only if the RPAA products were Smartamine M or Smartamine ML (as the efficacy of the protective coating technology and the bioavailability values of these products have been well documented (Rulquin and Kowalczyk, 2003; Schwab, 2003). For studies using Smartamine M and Smartamine ML, it was assumed that Smartamine M contained 75% Met and that Smartamine ML contained 40% Lys (accounts for 20% HCL content) and 15% Met. It was also assumed that 80% of the Lys and Met in each respective product was bioavailable. Studies in which AA were infused were included in the dataset if the aforementioned criteria were met. It was assumed that 100% of the infused AA were bioavailable.

All animal information reported in the published studies, such as BW, BCS, DIM, and lactation number was used when entering the diets into the NRC (2001) model. However, 25 diets from 5 experiments in 4 studies did not report BW; therefore, the default value of 650 kg from NRC (2001) was used. Of the 105 experiments used, 68 used multiparous cows, 2 used primiparous cows, 27 used both multiparous and primiparous, and 8 experiments did not provide parity information. As a result, default

parity values in NRC (2001) of fourth lactation cows 65 months old were used. Sixty experiments began in early lactation (<70 DIM), 40 began in midlactation (70 to 203 DIM) and 5 began in late lactation (>203 DIM).

Statistical Analysis

The Proc Reg procedure of SAS [Version 8.2 (2001); (SAS Institute, Inc., Cary, NC)] was used to examine the relationship between measured milk and milk protein yield and NRC (2001) predicted flows of MP, MP-Met and MP-Lys. Initially, the results of 464 NRC (2001) diet evaluations were included in the regression analysis. However, restrictions were imposed in an effort to eliminate studies from the dataset which the model predicted values of MP balance (g/d), RDP balance (g/d), and Lys and Met as a percent of MP which deviated widely from predicted requirements. The imposed restrictions were: 1) an MP-balance of the diets had to range from -300 to 100 g/d, 2) an RDP balance of the diets had to be greater than -200 g/d, 3) Lys as a percent of MP had to be less than 7.2% and Met as a percent of MP had to be less than 2.4%, 4) for plots based on MP-Met flows, diets had to have a Lys to Met ratio of greater than or equal to 3.0:1, and 5) for plots based on MP-Lys flows, diets had to have a Lys to Met ratio of less than or equal to 3.0:1. Four diets which contained Lys to Met ratios of exactly 3.0:1 were included in plots based on flows of MP-Met and MP-Lys because a ratio of 3.0:1 was considered to be co-limiting in both Lys and Met.

Restricting the dataset to include diets with a predicted MP balance of no greater than 100 g/d was done to exclude diets in which AA supply, including Lys or Met, may have exceeded requirements. The restriction to include diets with a predicted MP balance

of no less than -300 g/d was done to exclude diets, which were excessively limited in protein and could have led to excessive protein mobilization. Restricting the dataset to include diets with a predicted RDP balance of no less than -200 g/d was done to ensure that the nitrogen requirements of the microorganisms in the rumen were met. Restricting the dataset to include only diets which contained predicted Lys and Met concentrations in MP of less than 7.2 and 2.4%, respectively, was done to keep within the boundaries of the optimal maximum requirements for Lys and Met as predicted by NRC (2001).

RESULTS AND DISCUSSION

This study was designed to extend the preliminary work of Schwab et al. (2003; 2004) of predicting milk and milk protein yields from predicted flows of MP, MP-Met, and MP-Lys and to determine whether using the first limiting amino acid for milk protein production is more accurate than using MP to predict milk and milk protein yield.

The restrictions placed on the dataset were designed to remove diets which deviated widely from NRC (2001) predicted requirements. The restrictions were developed to allow for some inaccuracy in the NRC (2001) model's ability to predict flows of MP, yield of RDP, and content of AA in MP. However, there was a significant amount of confidence placed on the NRC (2001) model to accurately predict the results of the experimental diets. This is why the boundaries used for flows of MP, yield of RDP, and content of AA in MP are limited to what were believed to be normal ranges beyond which point the experimental diets used were considered to produce abnormal results.

To examine the impact each restriction had on the overall dataset, each restriction was removed one at a time while leaving the other restrictions in place and the overall number of diets added back into the resulting regression plots was observed. With all of the restrictions imposed, the resulting regression plots based on NRC (2001) predicted flows of MP contained 153 data points (Figure 4.1). When the restriction that the balance of MP had to be greater than –300 g/d as predicted by NRC (2001) was removed, only 20 diets were added back into the resulting plots and the R^2 and standard error were decreased from 0.81 and 3.04 to 0.76 and 3.28, respectively, for milk yield and from 0.89 and 69.09 to 0.84 and 83.27, respectively, for milk protein yield. This indicated that only 20 diets had an MP balance of less than –300 g/d. When the MP balance restriction of 100 g/d was removed from the model, 201 diets were added back into the regression plots and the R^2 and standard error were decreased from 0.81 and 3.04 to 0.38 and 5.25, respectively, for milk yield and from 0.89 and 69.09 to 0.48 and 142.08, respectively, for milk protein yield. When the RDP balance restriction of –200 g/d was removed only 27 diets were added back into the regression plots and the R^2 and standard error remained similar at 0.81 and 3.04 vs. 0.81 and 3.18, respectively, for milk yield and 0.89 and 69.09 vs. 0.90 and 69.90, respectively, for milk protein yield. When the restriction that Lys and Met concentrations in MP could not be greater than 7.2 and 2.4%, respectively, was removed from the dataset, only 12 studies were added back into the resulting regression plots and the R^2 and standard error remained similar at 0.81 and 3.04 vs. 0.83 and 3.01, respectively, for milk yield and 0.89 and 69.09 vs. 0.90 and 67.18, respectively, for milk protein yield. For plots based on MP-Lys and MP-Met, the additional restrictions that the Lys to Met ratio in MP had to be less than or equal to 3.0:1 for MP-Lys and had to be

greater than or equal to 3.0:1 for MP-Met were added. When these restrictions were loosened to include a Lys to Met ratio of less than or equal to 3.1:1 for MP-Lys and greater than or equal to 3.1:1 for MP-Met, 5 diets were added back into the MP-Lys plots and 11 diets were removed from the MP-Met plots. Interestingly, the R^2-values for the regression plots of milk protein yield vs. predicted MP-Lys flows decreased slightly when the Lys to Met ratio was increased from being less than or equal to 3.0:1 to less than or equal to 3.1:1 (0.94, SE = 62.78 vs. 0.93, SE 64.16). It appears that the NRC (2001) suggestion of the optimum Lys to Met ratio of 3.0:1 for maximizing milk protein synthesis is sensitive to increases in the ratio as small as moving from 3.0:1 to 3.1:1.

Schwab et al. (2004) entered the diet composition and production data of published studies into NRC (2001) and then used the results to develop prediction equations. The prediction equations were derived by regressing milk or milk protein yield on NRC (2001) predicted flows of MP, MP-Lys and MP-Met. The dataset based on supplies of MP was restricted so that predicted NE_L-allowable milk was greater than MP-allowable milk to ensure that energy was not limiting the responses in milk or milk protein yield. Another restriction imposed was that actual milk minus MP-allowable milk had to be within ± 6 kg/d of MP-allowable milk to help eliminate studies in which factors other than MP or energy limited lactation performance or where excessive protein mobilization may have been occurring. The dataset based on supplies of MP-Lys and MP-Met was restricted so that MP balance was between –250 and +100 g/d. A further restriction was imposed so that the Lys to Met ratio in MP was less than 3.25:1 for the dataset based on supplies of MP-Lys and greater than 3.0:1 for the dataset based on supplies of MP-Met. Their results indicated that flows of MP-Lys were the most accurate

at predicting milk and milk protein yield followed by MP-Met and MP. However, these diets may not have been limiting in MP-Lys. The restriction used for ensuring that the diets were limiting in MP-Lys was <3.25:1, which brings into question whether the diets were in fact Lys-deficient.

Doepel et al. (2004) conducted a more detailed and technical study using only published infusion studies with the goal of generating prediction equations for milk protein yield and changes in milk protein yield in response to changes in intestinal AA supply. Another goal of their study was to test the hypothesis that the efficiency of conversion of digestible AA into milk protein is not constant as is assumed by current models such as NRC (2001) and CNCPS (2000). The authors were unable to detect a significant relationship between the extent and direction of the change in milk protein yield and changes in total AA supply. As a result, regression equations predicting changes in milk protein yield in response to supplementary AA were not developed. However, they were able to demonstrate that the conversion of digestible AA into milk protein is not constant as is assumed by NRC (2001) and CNCPS (2000), but instead is variable. They concluded that by using variable efficiency coefficients, the ability to predict milk protein yield in response to additional supplemental protein would be improved.

Prediction Equations for Milk Yields and Milk Protein Yields

The plots derived by regressing milk and milk protein yield on MP, MP-Met and MP-Lys are presented in Figures 4.1-4.3, respectively. This study demonstrated that MP, MP-Met, and MP-Lys flows as predicted by NRC (2001) can be used to predict milk and

milk protein yield. In addition, this study demonstrated that MP-Lys was the most accurate predictor of milk and milk protein yield and milk protein yield is more accurately predicted by MP, MP-Met and MP-Lys than milk yield.

One of the most striking results of this study is the lack of diets which are deficient in MP-Lys as predicted by NRC (2001). A similar finding was observed by Schwab et al. (2004) where only 28 diets were included in the regression plots for milk and milk protein yield vs. MP-Lys (Figure 4.4). In that study, the Lys to Met ratio for diets considered deficient in Lys was even extended to 3.25:1. However, the resulting R^2-values of 0.90 and 0.92 indicate a strong association between milk and milk protein yield and MP-Lys flows when cows are fed Lys-deficient diets. In the current study, there was an even stronger association observed as indicated by R^2-values of 0.95 and 0.94 for milk and milk protein yield, respectively. The standard error was also lower for plots based on MP-Lys compared to MP or MP-Met for both milk and milk protein yield (1.86 vs. 3.036 and 3.82, respectively, and 62.78 vs. 69.09 and 98.87, respectively; Figures 4.1-4.3). This result would not be totally unexpected considering Lys is predominantly used for protein synthesis (Chung and Baker, 1992) as opposed to Met, which has a myriad of physiological functions. Because of the lack of diets which are deficient in Lys, it is difficult to determine the extent, if any, of adding more Lys-deficient diets to the dataset would improve the accuracy of predicting milk and milk protein yield based on MP-Lys flows. Regardless, as indicated by the results of this study and the results of Schwab et al. (2004), using MP-Lys flows is the best predictor of milk and milk protein yield compared to MP and MP-Met flows when Lys is first limiting or co-limiting with Met.

As previously mentioned, Doepel et al. (2004) observed that the efficiency of conversion of digestible AA into milk protein is variable and not constant as is assumed by models such as NRC (2001) and CNCPS (2000). It is important to note that the prediction equations developed in this study were not intended to be incorporated into the NRC (2001) model and only represent a more refined approach of the work conducted by Schwab et al. (2003) and Schwab et al. (2004). When examining the plots presented in Figures 4.1-4.3, it is evident that the data appears to follow a curvilinear pattern and not necessarily a linear pattern which confirms the results of Doepel et al. (2004). This study did not test for non-linearity and the authors recognize this deficiency. However, the goal of this study was to refine the results of Schwab et al. (2003) and Schwab et al. (2004) and now that this goal has been accomplished, new opportunities for conducting more complex statistical analyses on this dataset have been realized. Although the study conducted by Doepel et al. (2004) represents a more statistically complex analysis, the dataset used in that study was extremely limited. The dataset used in this study represents the results of a larger number and more varied research studies, which could be used to help further refine the results obtained by Doepel et al. (2004). Additional parameters to those used in the current study, such as stage of lactation, BW, parity, and breed could be included in future analyses to determine the effects these parameters have on the conversion of AA into milk protein.

CONCLUSIONS

This study demonstrated that MP, MP-Met, and MP-Lys flows as predicted by NRC (2001) can be used to predict milk and milk protein yield. In addition, this study demonstrated that MP-Lys was the most accurate predictor of milk and milk protein yield and milk protein yield is more accurately predicted by MP, MP-Met and MP-Lys than milk yield. However, this study was designed to further refine the results of previous attempts in our laboratory to predict milk and milk protein yield using NRC (2001) predicted flows of MP, MP-Met, and MP-Lys. It was not designed to develop prediction equations which could be incorporated into existing models, such as NRC (2001). The original idea for this effort was designed to help dairy nutritionists predict milk and milk protein yields when protein supplements such as, blood meal, protected soy products, and protected Met products were included in rations. Future research should focus on determining the variable conversion of MP-AA into milk protein to better predict milk and milk protein yield responses. In addition, stage of lactation, BW, parity, and breed effects could be included in future analyses to determine their impact on the conversion of AA into milk protein.

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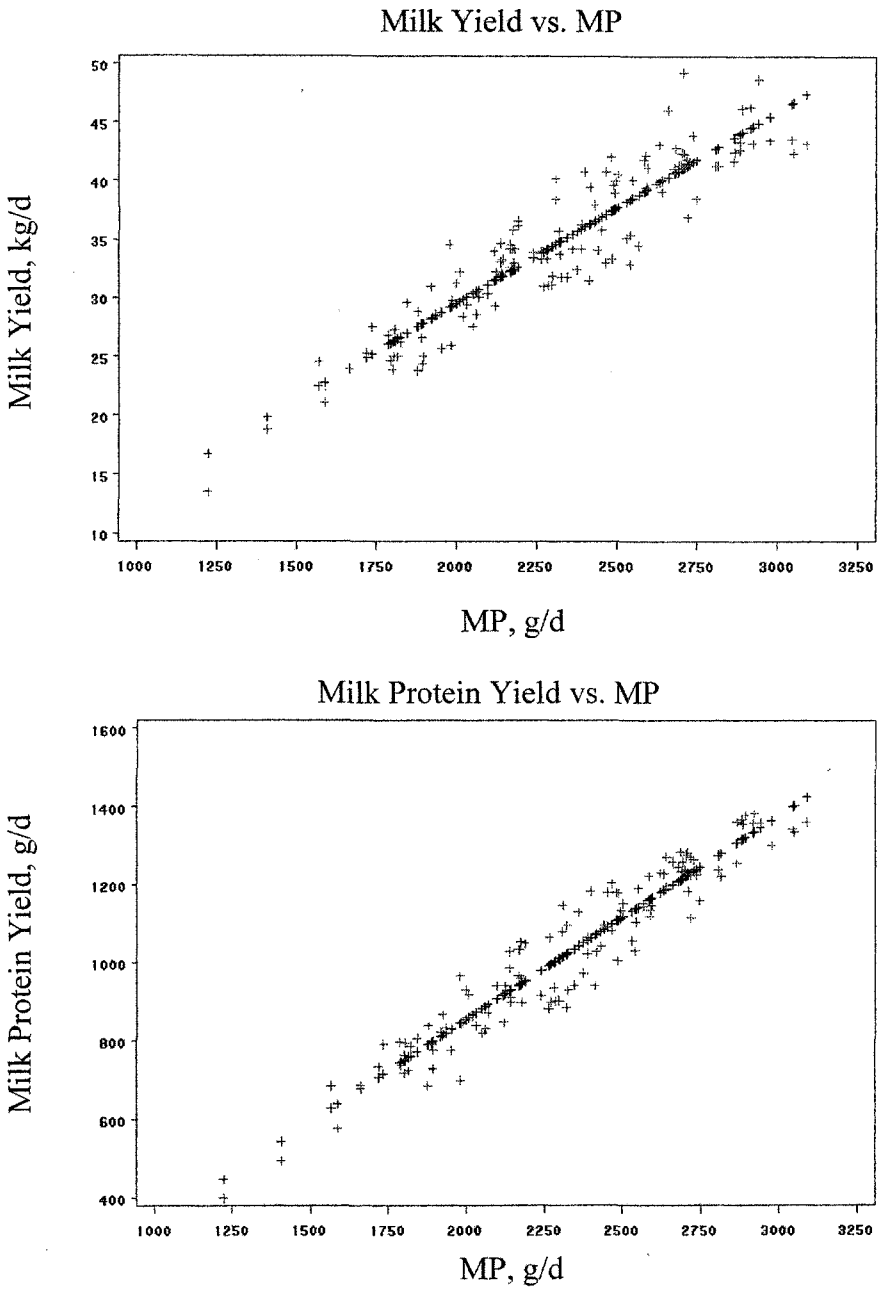

Figure 4.1. Plots of measured milk yields and milk protein yields vs. NRC (2001) predicted flows of metabolizable protein (**MP**) (g/d). Data used were selected from a database of 464 diets and were restricted to include only diets with an MP balance of -300 to 100 g/d, an RDP balance of greater than -200 g/d, Lys, % MP less than 7.2 and Met, % MP less than 2.4. The prediction equations for milk yields and milk protein yields, respectively, were (n = 153): y = -1.9003 + 0.0164x, R^2 = 0.8121, SE = 3.036 and y = -137.61 + 0.5183x, R^2 = 0.8934, SE = 69.087.

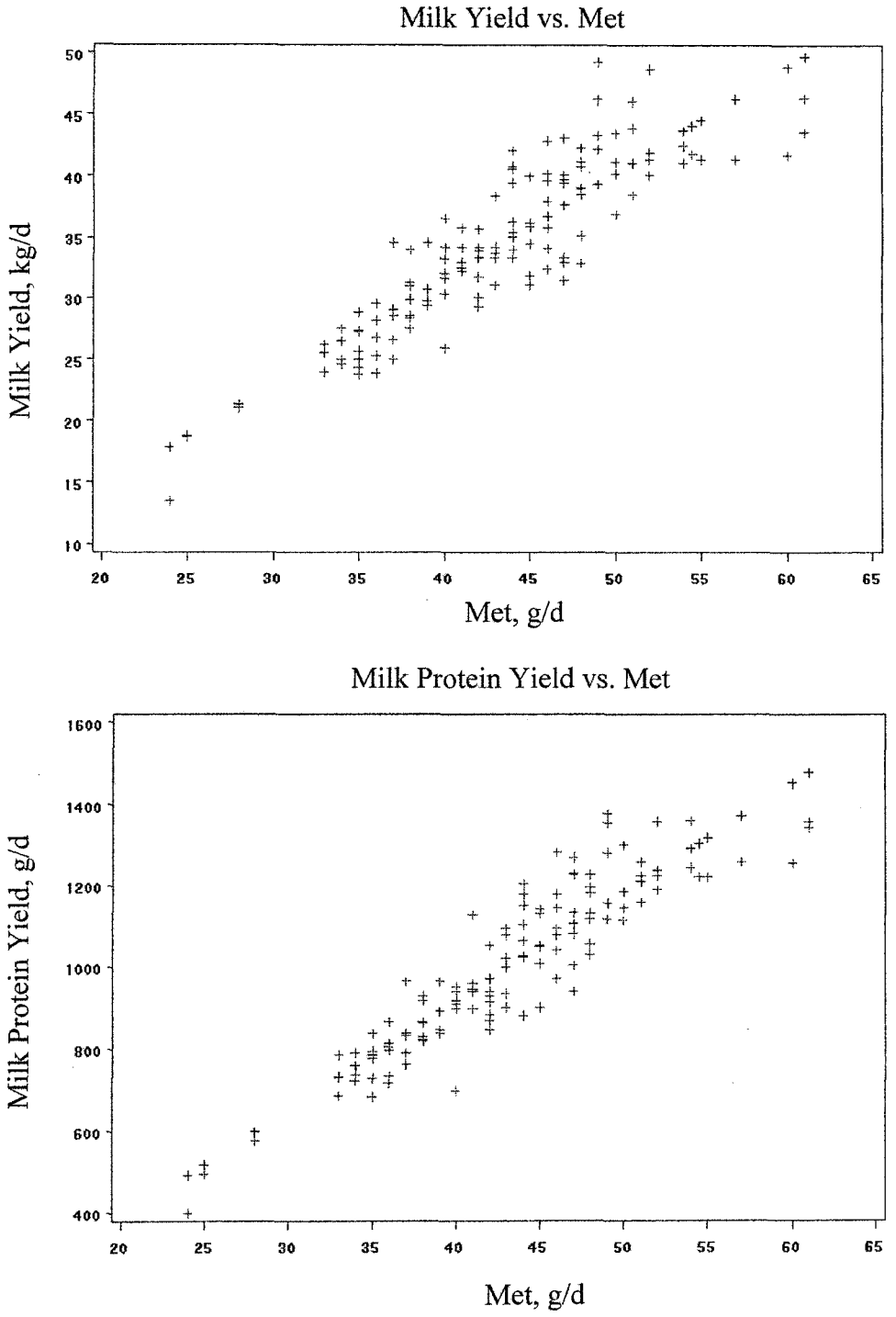

Figure 4.2. Plots of measured milk yields and milk protein yields vs. NRC (2001) predicted flows of metabolizable Met (g/d). Data used were selected from a database of 464 diets and were restricted to include only diets with an MP balance of -300 to 100 g/d, an RDP balance of greater than -200 g/d, Lys, % MP less than 7.2, Met, % MP less than 2.4, and Lys to Met ratio greater than or equal to 3.0:1. The prediction equations for milk yields and milk protein yields, respectively, were (n = 141): $y = 2.3205 + 0.7551x$, $R^2 = 0.6914$, SE = 3.815 and $y = -12.299 + 24.027x$, $R^2 = 0.7717$, SE = 98.873.

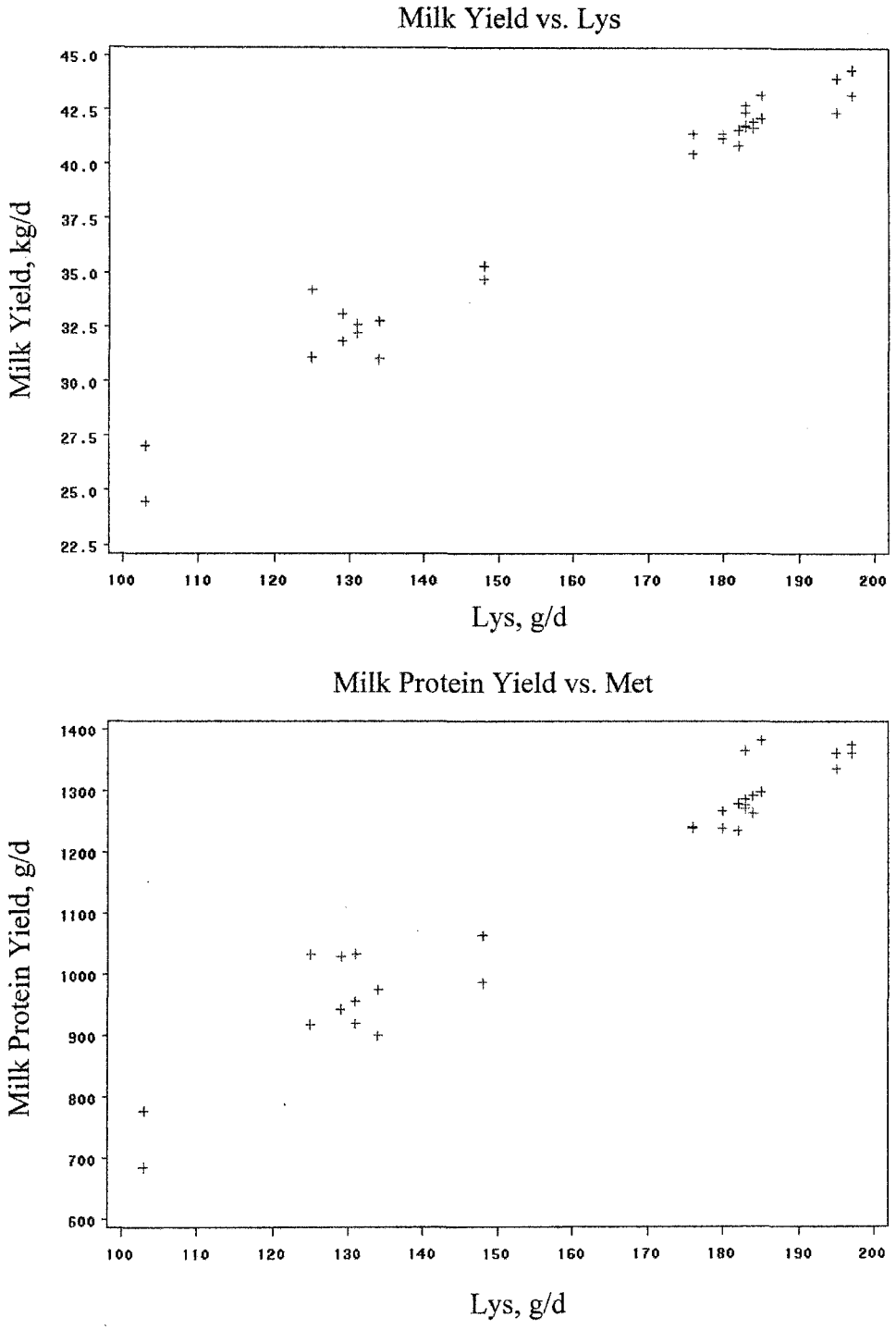

Figure 4.3. Plots of measured milk yields and milk protein yields vs. NRC (2001) predicted flows of metabolizable Lys (g/d). Data used were selected from a database of 464 diets and were restricted to include only diets with an MP balance of -300 to 100 g/d, an RDP balance of greater than -200 g/d, Lys, % MP less than 7.2, Met, % MP less than 2.4, and Lys to Met ratio less than or equal to 3.0:1. The prediction equations for milk yields and milk protein yields, respectively, were (n = 16): $y = 3.3641 + 0.2146x$, $R^2 = 0.9506$, SE = 1.8611 and $y = 91.461 + 6.564x$, $R^2 = 0.9406$, SE = 62.775.

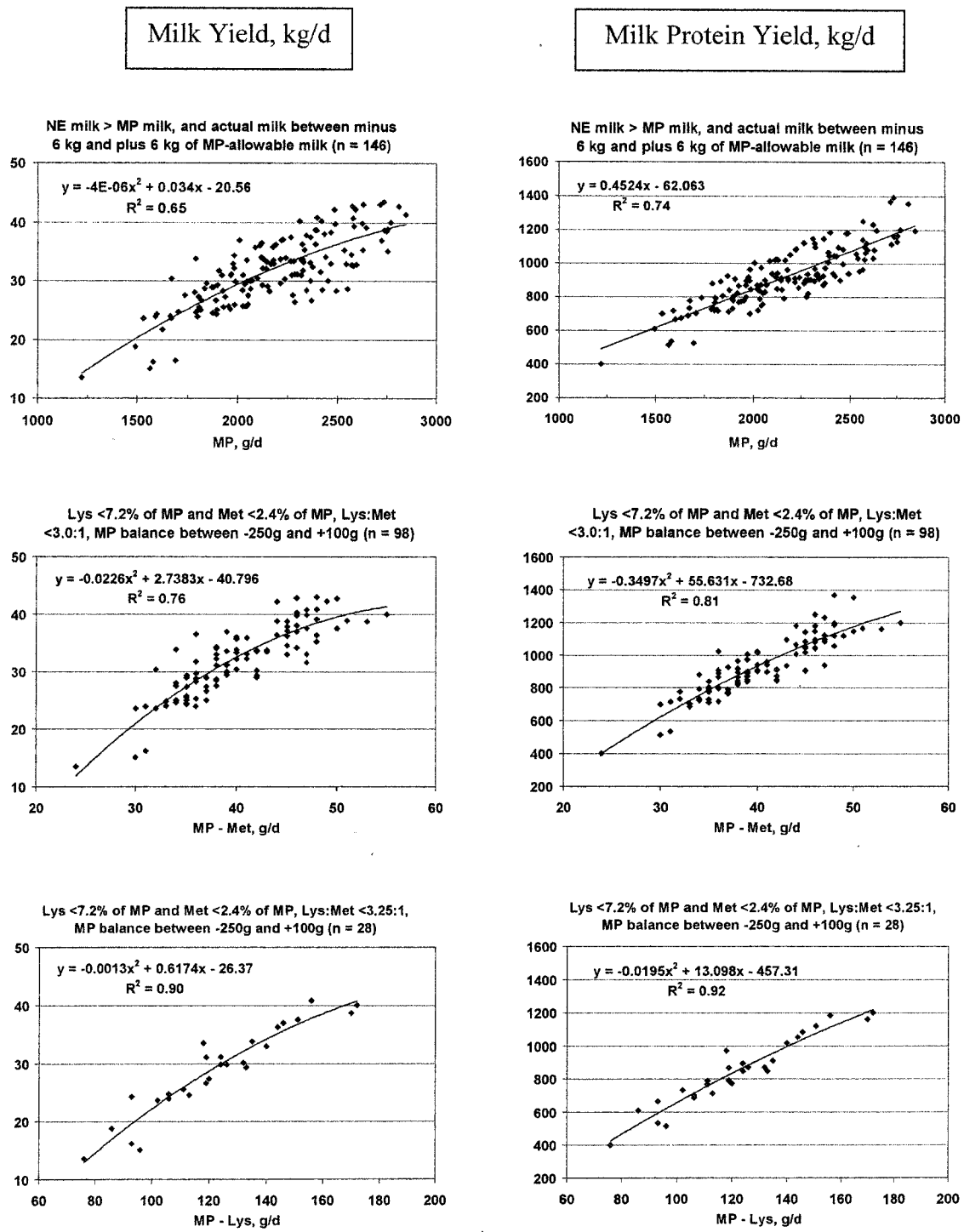

Figure 4.4. Plots of measured milk and milk protein yields vs. NRC (2001) predicted flows of metabolizable protein (MP) and MP–Lys and MP–Met. Data were selected from a database involving 321 diets fed to Holstein cows without AA supplementation (restrictions used for selecting data are indicated above each of the plots) (Adapted from Schwab et al., 2004).

APPENDIX

UNIVERSITY of NEW HAMPSHIRE

May 13, 2004

Schwab, Charles
Animal & Nutritional Sciences
Ritzman Nutrition Lab
Durham, NH 03824

IACUC #: 040403
Approval Date: 04/27/2004
Review Level: B

Project: Use of Changes in Plasma Methionine Concentrations to Measure the Effectivness of Methionine Products in Their Ability to Provide Absorbable Met to Lactating Dairy Cows Fed Met-Deficient or Met-Adequate Diets

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category B on Page 4 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the study involves either no pain or potentially involves momentary, slight pain, discomfort or stress.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this study. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:

1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Van Gould at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Roger Wells, D.V.M.
Vice Chair

cc: File

Research Conduct and Compliance Services, Office of Sponsored Research, Service Building,
51 College Road, Durham, NH 03824-3585 * Fax: 603-862-3564

UNIVERSITY OF NEW HAMPSHIRE

Office of Sponsored Research
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51 College Road
Durham, New Hampshire 03824-3585
(603) 862-3564 FAX

LAST NAME	Schwab	**FIRST NAME**	Charles
DEPT	Animal and Nutritional Sciences, Ritzman Laboratory	**APP'L DATE**	8/30/2001
OFF-CAMPUS ADDRESS (if applicable)	Animal and Nutritional Sciences, Ritzman Laboratory	**IACUC #**	010803
		REVIEW LEVEL	A
		TODAY'S DATE	9/12/2001

PROJECT TITLE Effects of Providing Supplemental Methionine in the Form of Smartamine M or HMBi (isopropyl ester of 2-hydroxy-4-methylthio butanoic acid) to Prepartum and Early Lactation Holstein Dairy Cows on Feed Intake and Lactational Performance

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All cage, pen or other animal identification records must include your IACUC Protocol # as listed above.

The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the protocol submitted for this study under Category A on Page 4 of the "Application for Review of Animal Use or Instruction Protocol" - the study involves only observation or normal maintenance of animals, or animals being held/bred but not yet used for research or teaching purposes.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please note: Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. *Participation is mandatory* for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Van Gould at 862-4629 or Julie Simpson at 862-2003.

For the Institutional Animal Care and Use Committee,

John A. Litvaitis, Ph.D.
Chair

cc: File
Ryan S. Ordway, Graduate Student