EVALUATING THE BIOAVAILABILITY OF RUMEN PROTECTED METHIONINE PRODUCTS AND THEIR EFFECTS ON PERFORMANCE OF DAIRY COWS

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Animal Science

Summer 2021

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ACKNOWLEDGEMENTS

I would like to thank my committee members for their help and support throughout this process. I would like to thank Dr. Gressley for taking me into her lab when I was uncertain in what direction to go after undergrad. She has been supportive, understanding, and a fantastic mentor throughout my time at the University of Delaware. I would also like to thank my committee members, Dr. Li and Dr. Kung for their guidance, and advice throughout this process.

I want to thank all the undergraduate students from our lab. I cannot imagine how I would have completed this research without you. From navigating late night and early morning shifts with the cows, milk sampling at 4 am, or arguing over trivia in lab while weighing out treatment’s you guys made it so that there was never a dull day. All of you kept things fun and on the right track, and I am extremely grateful.

I would like to thank our University of Delaware Dairy Crew. Without their hard work and dedication our research would not have been possible. I have learned so much from every one of them, and they have made my time out at the dairy a memorable one.

Finally, I would like to thank family and friends. You helped keep me pointed in the right direction and have been a constant source of support in my endeavors. Thank you for being there for me and encouraging me when I needed it most.
TABLE OF CONTENTS

LIST OF TABLES ........................................................................................................ vi
ABSTRACT .................................................................................................................. vii

Chapter

1. LITERATURE REVIEW .......................................................................................... 1
   1.1. Introduction ....................................................................................................... 1
   1.2. Utilization of Protein and Dietary Components ............................................... 3
   1.3. Modeling Nutrition Requirements .................................................................. 5
   1.4. Importance of AA ......................................................................................... 8
   1.5. Methionine’s Role in Energy and Fat Mobilization ....................................... 11
   1.6. Sources of Rumen Protected Methionine ..................................................... 13
   1.7. Effect of RPM supplementation on performance .......................................... 17
   1.8. Assessing bioavailability ............................................................................ 22
   1.9. Conclusion ................................................................................................... 27

2. OBJECTIVES ........................................................................................................... 29

3. EVALUATION OF PLASMA AMINO ACID RESPONSE OF A NEWLY
   DEVELOPED RUMEN PROTECTED METHIONINE PRODUCT TO TWO
   CURRENTLY MARKETED PRODUCTS IN HOLSTEIN DAIRY COWS ........ 31
   3.1. Introduction...................................................................................................... 31
   3.2. Materials and Methods .............................................................................. 32
   3.3. Results and Discussion .............................................................................. 36

4. EVALUATION OF ANIMAL PERFORMANCE RESPONSE TO TWO
   RUMEN PROTECTED METHIONINE PRODUCTS IN EARLY LACTATION
   HOLSTEIN COWS .......................................................................................... 45
   4.1. Introduction ................................................................................................... 45
4.2. Materials and Methods ................................................................................. 46
4.3. Results and Discussion ............................................................................... 51

5. CONCLUSIONS .......................................................................................... 57

TABLES ............................................................................................................. 58

REFERENCES .................................................................................................... 64

Appendix

IACUC PROTOCOL APPROVAL ......................................................................... 84
LIST OF TABLES

Table 1 Ingredient composition of experimental ration Experiment 1 ...................... 58
Table 2 Intake and production data for all cows Experiment 1 ................................. 59
Table 3 Plasma free amino acid concentrations (μM) Experiment 1 .......................... 60
Table 4 Ingredient composition ration Experiment 2 .............................................. 61
Table 5 Intake and production responses for all cows Experiment 2 .......................... 62
Table 6 Intake and production responses for multiparous cows Experiment 2 .......... 63
ABSTRACT

Two experiments were conducted to evaluate the performance of KESSENT M (RPM-K; Kemin Industries, Des Moines, IA, USA) to commercially available products by examining plasma methionine response and effect on animal performance in Holstein dairy cows.

For experiment 1, 10 multiparous Holstein cows 280 (± 73) days in milk (DIM) were used in a replicated 3x3 Latin square design with 7-day experimental periods. Treatments were administered at time of feeding three times daily and consisted of a control diet plus 12 g/d of one of three rumen protected methionine (RPM) products, either the newly developed product RPM-K or one of two existing products, RPM-S and RPM-M, with known differences in bioavailability. During days 5-7 of each period, blood samples were collected from jugular catheters at 2, 4, 6, and 8 h after the morning feeding, and underwent amino acid analysis. Plasma methionine data were analyzed using the full data set as well as theoretical data sets containing individual cow plasma samples pooled by day or period. We observed that plasma methionine was affected by treatment \((P = 0.006)\) and time \((P = 0.001)\). There were no differences between RPM-S and RPM-K (32.7 vs 33.0 \(\mu\)M, respectively; \(P = 0.79\)), and both were greater than RPM-M (30.1 \(\mu\)M; \(P \leq 0.001\)). The time affect was due to reduced plasma methionine at 4 h (30.2 \(\mu\)M) than at the 2, 6, and 8 h sampling times (31.9-33.0 \(\mu\)M; \(P > 0.05\)). Using the theoretical pooled plasma samples, differences observed in the full model were maintained when samples were
pooled by day, but only a trend for a difference among treatments was observed when samples were pooled by period. Bioavailability of RPM-K was similar to RPM-S and greater than RPM-M. Similar results would likely have been obtained had plasma samples been pooled by day for each cow in each period, but not if plasma samples had been pooled by period for each cow. 

For experiment 2, we utilized 24 multiparous and 6 primiparous Holstein cows 95 (±20) and 71 (±3) DIM, which were assigned treatments using a replicated 3 × 3 Latin square design with 21-day periods. Treatments consisted of a control diet deficient in metabolizable methionine (MP Met) by 17 g, or control diet plus 14 g/d one of two RPM products (RPM-K or RPM-S). Milk samples were collected on d 13-14 and 18-21 of each period. Plasma samples were collected at 2 and 6 hours after feeding on d 21 of each period and subjected to free amino acid analysis. Milk fat percentage of cows given RPM-S (3.73%) was not different from RPM-K (P = 0.32) and tended to be greater than Control (P = 0.0504). Milk fat yield was no different between RPM-K and RPM-S (both 1.48 kg/d; P = 0.78). There was no difference in milk protein percent between RPM-K and RPM-S (3.25%; P = 0.96), and both were greater than Control (3.22%; P = 0.02). Plasma free methionine as a percentage of total amino acids minus sulfur containing amino acids was no different between RPM-S and RPM-K (2.05 and 2.04, respectively; P = 0.87), and both were greater than Control (1.39; P < 0.0001). Both RPM-K and RPM-S demonstrated similar increases in milk fat and protein percent relative to the control. Plasma free methionine response to each supplement was similar, suggesting similar bioavailability.
Our objective was to evaluate the efficacy of the recently developed rumen protected methionine product KESSENT M to currently marketed products. There are limited options when it comes to viable sources of rumen protected methionine from which dairies can choose from, and it may be beneficial to expand upon competitive products. The efficacy of products was determined by their impact on plasma methionine and production performance in response to RPM supplementation. Our previous work had found that KESSENT M had a high bioavailability, and it was hypothesized that it would yield a plasma methionine and production response competitive to marketed products.
Chapter 1

LITERATURE REVIEW

1.1 Introduction

Modern dairy cows can produce vast quantities of high-quality milk due, in part, to a combination of nutritional management and an understanding of nutrient requirements. Amino acids (AAs) play an important role as they make up dietary protein which provides the animal with necessary building blocks for synthesis of proteins as well as alleviate stress and minimize the negative energy balance associated with lactation. Ensuring adequate nutrition, dairy operations typically spend more on feed than any other facet in maintaining the dairy. Reducing feed quality can help reduce feed cost, however cheaper feeds tend to be lower in nutrient quality and digestibility. These factors may negatively impact yield of a herd. Profitability is a balancing act between operating cost and milk income. To ensure optimum milk production within a herd, a dairy farmer must know what nutritional requirements are necessary for each stage of production and provide each group with a ration that can supply an appropriate balance of nutrients for maintenance, reproduction, and production to attain desired milk composition and production traits.

The incorporation of rumen protected amino acids (RPAA) into dairy diets has been used for years with success, particularly for meeting methionine requirements. Supplementing a diet with RPAA can reduce the amount of crude protein necessary in the diet. A reduction in crude protein (CP) can help alleviate cost when protein source prices
are high, which often can be the one of the more expensive additives. Another benefit of decreased crude protein level is possible reduction of nitrogen (N) waste from feed. As research is conducted on the use of amino acid supplements, great strides are being made in more accurate assessment of nutritional requirements, and a better understanding of how to maximize efficiency. Maximizing efficiency will both increase gross income of a dairy as well as reduce waste.

The major amino acid explored for its enhancements to dairy production is methionine. Methionine is usually the first limiting amino acid for milk production, and it plays an important role in milk protein and fat synthesis (Overton et al. 1998). Methionine is a sulfur containing AA which is involved in several synthesis pathways including synthesis of phospholipids, carnitines, creatine, and polyamines (Ardalan et al. 2011). Due to its involvement in these synthesis pathways as well as its role in milk protein and fat synthesis, sufficient quantities of available methionine are desirable to maximize production parameters and subsequently efficiency in dairy cattle. Benefits of supplemental methionine include increased milk yield and milk protein and fat content (Blum et al, 1999). Methionine is naturally occurring in feed presented at dairies; however, it is virtually impossible to meet methionine requirements using traditional feed components, requiring incorporation of rumen protected methionine (RPM) or a dramatic modification of the ration. Furthermore, availability and cost of protein supplements can be of concern at a dairy, leading to a desire to explore alternatives such as RPM supplements as potentially beneficial alternatives. RPM may be incorporated into a diet to
address methionine deficiencies or for substitution of a protein fraction within a diet to limit nitrogenous waste from feed (Yang et al 2010).

1.2 Utilization of Protein and Dietary Components

Lactating dairy cows have a high protein requirement due to the body's demand for amino acids to fuel milk protein synthesis. Metabolizable protein (MP) refers to the true protein digested post-ruminally which provides amino acids to the small intestine which are made available for metabolism and protein synthesis. For ruminants, this source of protein can come from high quality microbial protein which is synthesized by microbes within the rumen, or dietary protein which escapes rumen degradation, also known as rumen undegraded protein (RUP). Protein that is broken down within the rumen is known as rumen degraded protein (RDP), and it is responsible for providing necessary nitrogen to be incorporated into synthesized microbial crude protein (MCP). The microbes utilize the fermentable energy sources of the diet along with this N source to fuel protein synthesis. Rumen microbes can capture most of the ammonia released from AA deamination and the hydrolysis of non-protein nitrogen (NPN) compounds (NRC, 2001). The synchronization of rapidly fermentable and degradable starch and protein sources can stimulate microbial protein synthesis (Russel et al 1992). It has been observed that ruminal disappearance of ammonia and NPN increases with increased intake of fermentable carbohydrates due to microbial protein synthesis (Schwab and Broderick 2017). It is therefore understood that diets formulated to have ample levels of RDP and energy concentrations should result in greater microbial protein yields and greater MP for intestinal absorption to fuel milk protein synthesis.
Microbial crude protein can provide the appropriate amino acid profile for milk protein synthesis, though quantities of AA and MP may be insufficient in high producing cows (Virtanen, 1966). Microbial protein is synthesized by a mixture of rumen microflora which multiply within the rumen and flow to the lower gastrointestinal tract with the rumen digesta (Schwab and Broderick 2017). The microbial protein produced from these microorganism’s accounts for roughly 50% of the CP, which is supplied to the small intestines, accounting for a large portion of the AA supplied to the animals (Schwab and Broderick 2017). Microbial protein has generally been considered to provide good quality CP to dairy cows due to high apparent digestibility and balanced mix of amino acids (Schwab and Broderick 2017).

Dietary components will vary in their proportions of RUP and RDP dependent on type of feed and moisture composition. Typically feed components containing large amounts of moisture and protein concentrations, such as legume-based silages, can be expected to contain greater proportions of RDP (Krishnamoorthy et al, 1983). Feeds containing low amounts of moisture due to drying and processing can be expected to contain greater concentrations of RUP. Traditionally, RUP is supplied in diets to provide more essential amino acids (EAA) directly to the animal rather than the rumen microbes to further performance in lactating animals. Cows which have elevated feed intake tend to also have a higher RUP proportion in their diet due to more rapid feed passage through the rumen. Early lactation dairy cows which have large RUP requirements and low feed intake can benefit from supplemental rumen bypass products as it will ensure a more desirable array of amino acids to the lower digestive tract during a period of high stress and milk
yield (Maiga et al., 1996). These early lactation cows may not be able to consume adequate amounts of feed to ensure adequate levels of microbial protein synthesis or a proper balance of amino acids supplied to the mammary gland for milk protein production. Though both RDP and RUP are important for ensuring strong lactational performance, the RUP fraction is a more accurate representation of amino acid profile which passes the rumen and is made available to the cow for milk production (Maxin et al 2013).

The rumen is a unique compartmentalized structure which facilitates the utilization of cellulose and NPN to produce high quality rumen MP through microbial degradation (Maiga et al., 1996). The rumen can produce protein to drive production from low quality protein sources. In general MP from the rumen is capable of supplying all necessary AA for milk protein synthesis, however the protein supply may not provide sufficient quantities of AAs on a g/d basis which may limit milk and milk component yields in high producing cows (Papas and Wu 1997, Nimrick, 1970).

1.3 Modeling Nutrition Requirements

Accurate predictions of protein supply of dairy cows continue to be an area of focus and research in the dairy industry. Crude protein has been generally defined as nitrogen content x 6.25, which is based on the understanding that proteins on average contain 16% N (NRC, 2001). Reliance on crude protein as a measure when evaluating amino acid supply to the animal congregates all nitrogen containing components into one measure which may lead to greater variability in the evaluation of an animal’s supply of amino acids (Ipharraguerre and Clark, 2005). The CP fraction of feedstuffs is made of multiple fractions which experience ruminal disappearance at differing rates as the result of degradation and
passage (NRC, 2001). The National Research Council (NRC) protein requirements were established using a mechanistic system developed from in situ data to calculate the RUP and RDP content of feedstuffs. Regression equations of this system allow for estimates of how RDP and digestible organic matter affect MCP production, and predicting changes in RUP based on dry matter intake (DMI), percent concentrate feeds in diet dry matter (DM), and percent NDF of diet DM, along with passage rate (NRC, 2001). In doing this they were able to improve upon previous models of the NRC 1989, however it was understood that changing to balance for AAs as a percentage of MP would be a more accurate model. The NRC opted to ignore the inclusion of AA as a percent of MP into its model as at the time the committee believed it lacked data to accurately put forth a model to quantify the AA requirements for dairy cattle (NRC, 2001). What was needed to create an AA model was an accurate understanding of net AA requirements for maintenance, growth, pregnancy, lactation, the AA composition of products, and an understanding of the partitioning of absorbed AA for maintenance and product formation (NRC 2001).

Due to this understanding, and studies showing improvements to AA profile of MP can improve milk protein and yield while simultaneously reducing excess dietary protein, a move from CP to AA based ration balancing occurred. Production responses of lactating dairy cows to increased lysine and methionine in MP showed variable increase in milk protein, milk yield, and feed intake, with Schwab et al. (2005) reporting MP lysine and methionine as predictors of milk volume and milk protein yield. Methionine supply appeared to be a better predictor of milk volume (R² of .76) and milk protein yield (R² of 0.81) than MP alone (R² of 0.65 and R² of 0.74). This showed that predictability of animal
performance could be improved by formulating rations by the first limiting AAs, however this required an amino acid profile library of feedstuffs and estimates of microbial AA supply (Schwab et al, 2005; Piepenbrink et al, 1996; Nocek et al, 1988).

This better understanding of MP requirements along with EAA requirements, particularly methionine and lysine, along with kinetics resulted in nutritionists developing the Cornell Net Carbohydrate Protein System (CNCPS) (Russel et al, 1992; Higgs et al, 2015). This model breaks CP into five fractions consisting of NPN, three different true protein fractions determined by fractional rate of degradation, and the final fraction is characterized by indigestible proteins associated with lignin, tannins and other heat damaged proteins (Higgs et al, 2015). This early iteration CNCPS model was based upon the information accumulated from the Nutrient Requirements of Beef Cattle (NRC, 2001). For ration balancing, it is important to identify the level of absorbed AA required for milk protein synthesis and body protein synthesis while minimizing AA waste (Higgs et al, 2015). The CNCPS utilizes a feed library of approximately 800 ingredients, along with data from NRC publications and CPM-Dairy, a ration formulation and evaluation program (Higgs et al, 2015). Early CNCPS models expressed AA as a percent of buffer insoluble residue, however current models express AA on a percent of MP basis (Higgs et al, 2015; O’Connor et al., 1993).

More recent efforts have focused on predicting amino acid requirements relative to dietary energy, a strategy that is employed in monogastric nutrition. Through analyzing studies in which AA was infused into the abomasum, duodenum, or intravenously, Higgs et al. (2015) applied these data sets to determine the efficiency of use for each AA and
impact of energy supply on AA utilization. Utilizing this model, Higgs identified that expressing AA supply relative to MP yielded a definitive relationship, however when expressing AA relative to metabolizable energy (ME), a stronger relationship was observed. LaPierre et al. (2019) applied Higgs work when comparing performance between a neutral diet balanced according to the previously calculated optimal AA to ME ratio (14.5% CP) (Higgs, 2015) and a diet formulated to be one standard deviation greater than this neutral diet (16%), and found that despite a 200 g difference in MP supply, both diets yielded similar performance results (LaPierre et al, 2019). This finding supports that MP does not adequately describe nitrogen requirements for lactating dairy cows and that greater accuracy can be achieved by discussing amino acids in terms of ME (LaPierre et al, 2019).

1.4 Importance of AA

Inclusion of rumen protected amino acids (RPAA) into a diet can alleviate deficiencies in the ration as well as be used for precision feeding. Precision feeding of limiting AAs has been shown to effectively increase DMI, milk yield, and milk protein concentrations (Zhou et al 2016). Protein-deficient or limiting diets have the capacity to limit production if not properly balanced due to decreased supply of essential AAs, resulting in reduced volume of milk produced and protein percentage. A properly balanced diet can achieve the same production parameters as one which overfeeds protein, as excess protein does not necessarily translate to an increase in milk protein yield past what is genetically possible for an animal (Maiga et al., 1996). It can be difficult to balance for AAs in ruminants due to variability in AA content of feed stuffs, difficulty in quantifying
microbial contribution to the small intestine, rumen degradation, glucogenic use of AA, and differences in AA concentration of milk and the extraction of AAs by the mammary gland (Schwab and Broderick., 2017).

In the US, the primary energy source for total mixed ration (TMR) comes from corn-based feeds, and the primary supplemental protein source is soybean meal. When incorporated together with other feedstuffs, nutritional requirements normally balance, however diets whose protein sources utilizes large amounts of corn and soybean meal may experience deficiencies in lysine and methionine with high producing cows. Protein from feed in conjunction with MCP may not be enough to make up for inadequacies in AA content. Lysine and methionine concentrations are quite low in most feeds, and lower than that of MCP. Microbial crude protein accounts for roughly 50% of the CP, which is supplied to the small intestine, of which 80% are AAs (Schwab and Broderick., 2017). The remainder of CP and subsequently AAs necessary for production are acquired from the ration. The lysine and methionine content of milk is similar when compared to that of microbial crude protein (16.4 vs. 15.9 %; 5.1 vs. 5.2 % of the total EAA for Lys and Met respectively) (Schwab and Broderick., 2017). Protein supplements on the other hand tend to not match these EAA requirements, with soybean meal containing 13.0 and 2.9 %, blood meal containing 17.5 and 2.5 %, brewers grains contain 6.7 and 4.5 %, and corn gluten meal containing 3.8 and 7.2 % Lys and Met respectively (Schwab and Broderick., 2017).

Methionine has been observed to be the first limiting amino acid in lactating dairy cows since the 1960s, after which attempts were made to develop rumen protected supplements (Schwab and Broderick 2017). Methionine supplementation has been shown
to benefit high producing dairy cows in production, milk protein, and milk fat. Methionine does not appear to have one specific route by which it is utilized and directly drives milk production, but rather it serves as an important building block through the donation of raw materials for synthesis of other key amino acids as well as its incorporation into various lipoproteins (Ardalan et al, 2011). Methionine is involved in many related pathways, including the synthesis of carnitine, creatine, polyamines, and phospholipids. Met is an important supplier of sulfur groups for synthesis of deficient NEAA such as cysteine and for inclusion in protein. Met plays important roles in protein synthesis, donation of methyl groups for reactions and donation of its sulphur groups for cysteine synthesis (Ardalan et al 2011). Since Met is able to be used to synthesize a variety of key milk components, its supplementation into a diet can help improve milk yield. Observed increases in milk fat synthesis may be due to methionine’s importance as a methyl donor for lipid biosynthesis and its involvement in transmethylation (Robinson et al, 1998). There are several ways in which methionine may improve milk protein synthesis. In addition to serving as a direct building block for milk protein, supplemental methionine improves insulin activity and availability of energy, upregulates L-type amino acid transporter 1 (LAT 1) of the mammary gland, and can provide sulfur groups for synthesis of cysteine (Ardalan et al, 2011).

Dairy cows obtain their amino acids through the breakdown of RUP and RDP. The RUP fraction of enters the digestive tract, passes through the rumen to the intestines. At the small intestine proteins are broken down into fragments and individual AAs by pancreatic enzymes for absorption (Arriola et al., 2014). Proteins are broken into
tripeptides, dipeptides, and AAs after which they can be absorbed across the lumen of the duodenum or jejunum of the small intestine via active transport. Amino acids, and other macronutrients are then transported through the body via the hepatic portal vein, where ultimately, they arrive at the liver for further breakdown and distribution as needed. The RDP fraction is utilized for the synthesis of MCP. Rumen degradable protein enters the rumen where microbial enzymes degrade protein into individual AAs, and ammonia for use by rumen microbes. Rumen microbes make use of ammonia, carbon, and energy from fermentable carbohydrates to synthesis AAs and MCP, which passes to the small intestine, following the same route as RUP. Excess amounts of ammonia which are produced by the catabolism of AAs, are absorbed to the bloodstream and transported to the liver where it is converted to urea and either exerted in urine or recycled in the animals saliva to be used as a NPN source in the rumen (NRC, 2001).

1.5 Methionine’s Role in Energy and Fat Mobilization

Dairy cattle experience a negative energy balance as they transition from gestation to early lactation due to reduced energy intake and elevated energy demand. Cows can adapt to this stress by mobilizing adipose tissues and hydrolyzing triacylglycerol (TAG) to non-esterified fatty acid (NEFA) and glycerol (Grummer, 1995; Karimian et al., 2015). This mobilization process is promoted by an increase in insulin resistance within the adipose tissue resulting in greater glucose availability and a decrease in pancreatic insulin secretion (Bell, 1995). The mass mobilization of material is important for use as energy and for milk fat production during early lactation. NEFAs are metabolized via the liver to produce very low-density lipoprotein (VLDL), re-esterified to TAG, completely oxidized
into adenosine triphosphate (ATP), and carbon dioxide, or partially oxidized to ketones (Donkin, 2012). Very low-density lipoproteins are a type of lipoprotein which allow for the transport of fatty acids through the bloodstream to tissues and if suppressed can limit lactational performance. As the capacity of the liver to metabolize NEFA to TAG is reached, TAG and acetyl CoA not utilized by the tricarboxylic acid cycle (TCA) may be converted to ketone bodies (acetone, acetoacetate, and beta-hydroxybutyrate) which can appear in blood, milk, and urine (Grummer, 1993; Donkin, 2012). Non-esterified fatty acid oxidation increases acetyl-CoA which can support ketogenesis, while re-esterification of NEFA to TAG can integrate with apoproteins to be exported as VLDL. Limited release of TAG into blood in early lactation cows can result in lipid deposition, putting animals at risk for ketosis or fatty liver (Kлепpe et al, 1988). The liver plays a crucial role in extracting NEFA but can progress to fatty liver due to TG accumulation (Ardalan et al 2011). Ketosis is the result of large concentrations of ketone bodies released into the bloodstream associated with excess fat mobilization seen in early lactation. Fatty liver often is seen in over conditioned cows at calving or just before parturition as a decrease in feed intake occurs. The mobilization of fat reserves can lead to excess intracellular TG accumulation in liver cells leading to cell damage and impaired function. This accumulation in the liver can lead to a decline in milk production, metabolic disease, and decrease in animal performance.

Methionine has been found to improve the ability of mobilized fats to fuel production, limiting fatty liver during the transition period, as well as supplying the building blocks that drive performance and milk production. Methionine is important in
the synthesis of phosphatidylcholine (PC) and related compounds, which package VLDL for transport. Supplementation may increase the availability of Met for lipoprotein synthesis to improve liver TG clearance as VLDL (Ardalan et al 2011). The NRC (2001) recommends a duodenal supply Met percent of 2.4% of MP. This is achieved by modulating the synthesis of s-adenosylmethionine during the methionine cycle. S-adenosylmethionine acts as a key methyl donor during the synthesis of PC from phosphatidylethanolamine (Osorio et al., 2014). Additional metabolic roles that methionine plays other than protein synthesis include trans-sulfation and methylation reactions to produce cysteine (Brosnan and Brosnan, 2006) and choline synthesis (Emmanuel and Kenelly, 1984). Methionine can support synthesis of choline and betaine metabolites. Choline plays an especially important role during the periparturient period and early lactation when there is a large mobilization of fatty acids of the adipose tissue.

Methionine can ensure sufficient metabolites are available to promote proper liver function during the transition phase in dairy cows. One indicator of impaired liver function post calving is elevated aspartate aminotransferase (AST) levels. This impairment may be a result of triglycerides infiltrating hepatocytes leading to cell damage. Studies by Ardalan et al (2011) reported a significant reduction of AST levels in postpartum cows with RPM supplementation. Similarly, Krober et al., (2000) saw a significant reduction of plasma AST in early lactating dairy cows given RPM.

1.6 Sources of Rumen Protected Methionine

Early efforts in rumen bypass practices involved heat or chemical treatments of proteins. These techniques primarily focused on limiting the breakdown of protein by
microbial enzymes in the rumen to ensure desired amino acid profiles were supplied to the animal (Papas and Wu 1997). For example, the intraruminal administration of casein resulted in poor N retention in sheep, however heat-treated casein produced less ruminal ammonia and increased N retention (Chalmers et al, 1954). Untreated proteins can be extensively degraded within the rumen to produce dialyzable peptides, free AA, and finally ammonia. Heat and chemical treatments proved successful as a low-cost option for reducing solubility in the rumen. Chemical treatments, such as formaldehyde, though effective at surviving the rumen, gave rise to health and safety concerns due to the nature of these chemical treatments (Schwab, 1996). Maillard reactions for instance, which occur at elevated temperatures, will reduce bioavailability of essential amino acids due to the formation of Maillard reaction products which may be unavailable for utilization by the body (Van Rooijen et al, 2013). Heat treatments remain one of the principal methods for limiting ruminal degradation of feed proteins and remains widely used to improve the RUP of soybean meal, whole soybeans, and other protein supplements for dairy cows (Schwab, 1996; Broderick et al, 1990).

Though treated proteins can be and have been used to increase the supply of post ruminal protein supply in dairy cows, it remains difficult to use those sources to meet EAA requirements. This gave rise to further investigation of the strategic supplementation of limiting RPAA through two main protection strategies of either polymer or lipid coatings. Research has focused heavily on the development of synthetic polymer coatings which were resistant to both microbial and mechanical degradation experienced within the rumen. Individual AAs or AA mixtures could be embedded into polymers and administered in
feed. These polymers were designed to bypass rumen microbial degradation and then release AAs under the acidic conditions found within the abomasum, resulting in an improved AA profile provided to the animal (Papas et al, 1984). Due to concerns of polymer residues posing possible negative health effects and subsequent commercial viability concerns, groups began exploring lipids as an alternative vehicle for avoiding rumen degradation (Chalupa and Sniffen, 1996). Lipid based products possess poor solubility in the rumen and are resistant to rumen microbial enzymes but can be broken down by mammalian enzymes found in the small intestine. Lipid coatings have the advantage of utilizing low-cost food grade materials to deliver targeted AA to the intestine (Papas and Wu, 1997). Some challenges faced by lipid matrices include a trend for reduced release of product to the animal compared to polymer coatings due to decreased carrying capacity of these newer technologies as well as the challenge of an inverse relationship between rumen survivability and release of active materials in the intestine (Papas and Wu, 1997). The reduced payload of AA within the lipid coating means more product is necessary to achieve similar results to polymer coatings.

Over the past several decades, a variety of lipid and polymer coated methionine products have been introduced to the market including Ketionin (Delmar Chemicals, LaSalle, QC, Canada), Met-Plus (Nisso America, Inc), Mepron (Evonik Industries, Hanau, Germany) and Smartamine M (Adisseo USA Inc., Alpharetta, GA). Smartamine M and Mepron have been some of the most successful of these products having been extensively studied for rumen survivability, intestinal digestibility, and effect on animal performance (Schwab, 2004). Meta-Smart (Adisseo USA Inc. Alpharetta, GA), is a source of available
Met for dairy cows which is provided as an isopropyl ester of 2-hydroxy-4-(methylthio)-butanoic acid (HMTBi) and is capable of being supplied in a pelleted form (57% HMTBi), or liquid formula (95% HMTBi). HMTBi functions differently from RPM products in that approximately 50% is absorbed across the rumen wall as metabolizable Met for the cow, and 50% is used in the rumen by rumen microorganisms. Another Met analogue which has been used alongside HMTBa is 2-hydroxy-4-(methylthio)-butanoic acid (HMTBa). Both HMTBa and HMBTi are subject to rumen microbial metabolism, providing rumen microorganisms with a source of Met (Vázquez-Anon et al., 2001). Both HMTBa and HMBTi are believed to primarily improve animal performance through improvements to rumen microorganism’s activity (Martin et al., 2013).

Mepron (Evonik Industries, Hanau, Germany), is a commercial product which has found success in delivering a rumen stable delivery system. Mepron supplies 85% DL-Met with small amounts of starch, fat, ash and a coating of ethyl cellulose, and stearic acid film (Schwab, 1996). The resultant slow release lipid-coated product contains a high payload of DL-Met in a pelleted product resistant to rumen degradation and available for release at the intestines (Waterman et al, 2012). Smartamine M utilizes a lipid/pH-sensitive copolymer to protect its 75% DL-Met core. Smartamine M releases DL-Met in response to the lowering of pH, such as that found in the abomasum. Smartamine M uses an ethyl cellulose coating with an additional poly (2-vinylpyridine-co-styrene) polymer added to the stearic acid, giving its coating the unique pH sensitivity and bioavailability of 80% (Graulet et al, 2005). Kessent M, a more recently designed RPM, utilizes a similar pH sensitive copolymer coating around a DL-Met core making it pH sensitive. Kessent M
makes use of a vinyl pyridine/styrene copolymer which resists degradation by the rumen, releasing Met as it reaches the acidic environment of the abomasum.

1.7 Effect of RPM supplementation on performance

The supplementation of postpartum and early lactation dairy cows with RPM has yielded a mixed batch of results on DMI largely dependent upon source or encapsulate technology. Studies such as those by Overton et al (1996), and Lara et al (2006) reported that there was no effect of RPM on DMI. Socha et al., (2005) saw a trend ($P > 0.15$) for a decreased DMI postpartum when feeding the RPM product Smartamine M but no effect in other protection technology. Strzetelski et al., (2009) and Ordway et al., (2009) observed no effect in mean DMI across their study when feeding RPM. A meta-analysis by Zanton et al., (2014) noted dry matter intake effects in response to Met supplementation varied between sources. Rumen protected methionine supplementation where the source was Smartamine M resulted in consistent increase in DMI, while products such as Mepron (Evonik Industries) produced no significant DMI effect (Zanton et al, 2014).

Rumen protected methionine supplementation’s effect on milk yield remains inconsistent, varying between experimental design and encapsulation technique. Ordway et al., (2009) and Overton et al., (1996) both reported that milk yield was unaffected when diets were supplemented with 20 g/d of RPM. Effect of RPM on milk fat remains unclear as there is not always an increase, with seemingly just as many studies observing increases as no effect at all. Differences observed in these studies may be in part due to encapsulation of Met products or diet composition (Yang et al 2010; Zhou et al, 2016). There is evidence that the maximum production response to RPM supplementation may be achieved with
dosages between 15 and 16 g/d with products containing approximately 80% DL-Met (Lara et al., 2006; Guinard and Rulquin, 1994; Armentano et al., 1997). Lara et al (2006) reported improvement to milk production ($P<0.01$) with RPM supplementation improving milk yield quadratically ($P<0.05$) to dose with a maximum yield at 16.2 g/d of RPM product. Overton et al (1996) reported increased yields of 3.5% after supplementation with 20 g/d of an RPM product containing 85% DL-Met when given within the first 126 days of lactation.

The addition of RPM to the diet has commonly been shown to improve milk protein percentage (Zanton et al., 2014). Socha et al (2008), observed a linear increase in milk protein percentage when supplementing mid-lactation cows with increasing amounts of duodenal infused Met, ranging from 0-20 g/d, resulting in milk protein percent increase from 3.22% at 0 g/d to 3.32% at 20 g/d DL Met. The mechanism by which RPM supplementation improves milk protein percentage appears to differ across commercial products and coating technology. In their meta-analysis, Zanton et al., (2014) reported that RPM products Smartamine and Mepron produced similar milk protein concentrations to that of post-ruminally infused DL-Met. Both Mepron and Smartamine M increased milk protein concentration by 0.07 % on average and DL-Met increased milk protein concentration by 0.08 % respectively. Zanton et al., (2014) also examined papers where supplementation of HMTBa was examined and found it provided no improvement to milk protein concentration. Lara et al (2006) reported there was a linear increase in milk protein percentage from 3.35 % in the basal diet to 3.52 % in response to supplementation of 0, 8, 16, and 24 g/d RPM product Mepron (85% DL Met). A lactation study by Armentano et al
(1997) reported linear increases in milk protein percentage from 2.89 to 3.01% when supplementing 5.25, 10.5, and 11.5 g/d DL-Met from Smartamine M. Another linear response was reported by Ardalan et al (2021) with protein percentage increasing when cows were supplemented with 0, 7.5, and 15 g/d RPM Smartamine M. Milk protein percent increase from a basal level of 2.77 % to 2.87% when supplemented with 15 g/d of Smartamine M (Ardalan et al., 2021). Ordway et al (2009) reported improved milk protein response to two RPM products (Smartamine M, 75% DL-Met; MetaSmart, 70%), producing 2.87 % and 2.81 % milk protein respectively compared to the control of 2.72 %. In their work with high producing dairy cows, Overton et al (1996) reported an increase in milk protein percent from 0.438 to 0.439 % when cows were supplemented with 20 g/d Mepron primarily due to increased synthesis of casein in response to RPM feeding. This increase in casein reflected increases in CP and true protein in milk with whey protein unaffected by RPM treatments (Overton et al 1996). Milk protein percentage tends to have a strong linear response to RPM supplementation, with improvements of around 0.2 percentage units per gram of MP Met.

Due to the increased milk yield and milk protein percentage seen with Met supplementation, increases in milk protein yield is to be expected. Increase in milk protein yield in response to RPM supplementation is one of the most observed responses (Rulquin et al, 1993; NRC, 2001; Zanton et al., 2014). Lara et al (2006) observed a quadratic response ($P<0.05$) in milk protein yield 1094, 1187, 1252, and 1166 g/d (0, 8, 16, and 24 g/d RPM product Mepron respectively) to supplementation of RPM. This quadratic response agreed with results from Guinard and Rulquin (1994) and Papas and Wu., (1997).
This quadratic response has been associated with increased synthesis of casein and reduced milk urea nitrogen (Papas and Wu., 1997). In contrast, Ardalan et al., (2021) reported a linear response to RPM supplementation of 7.5 or 15 g/d of Smartamine M (P = 0.004), with milk protein increasing to 1.33 kg/d and 1.31 kg/d, respectively, from a control of 1280 g/d in Holsteins receiving diets deficient in metabolizable Met. Similarly, Armentano et al (1997) observed a linearly increase in milk protein when supplementing Smartamine M (5.25, 10.5, and 11.5 g/d DL-Met) resulting in an increase in milk protein by 4 g for each 1 gram of Met added to the diet. Socha et al (2008), found a 7 % increase in milk protein could be attributed to supplementation of RPM (16 g/d) alongside lysine (10 g/d) from 10 to 50 DIM. Zhou et al (2016) reported that supplementing cows with Smartamine M supplemented at 0.08% DM of diet, increased milk protein yield from 1.25 kg/d in the control to 1.43 kg/d. A meta-analysis of RPM on performance by Patton (2010) reported an average milk protein yield increase for Smartamine M and Mepron of 13 g/d to 35 g/d respectively. Zanton et al (2014) noted that cows infused with DL-Met or supplemented with Smartamine experienced a 19 g/d increase in milk protein yield compared to control cows (P < 0.05), while Mepron on average increased milk protein yield by 35 g/d per gram of supplied Met. It's estimated that for every 1 g of MP Met an increase in milk protein of 2.3 g can be expected (Zanton et al., 2014). Milk protein responses of various Met sources were not significantly different from post ruminally infused DL-Met (P > 0.05) (Zanton et al, 2014), indicating that the RPM supplements are efficacious.

Improvements to milk fat yield through RPM supplementation have been observed though margins for improvement have varied. Fatty acids (FA) involved in milk fat
synthesis can be manufactured through de novo synthesis in the mammary gland or uptake from circulating long chain fatty acids (LCFA) (Neville and Picciano, 1997). Methionine's involvement in the transference of methyl groups in the form of S-adenosylmethionine, may be the method by which RPM supplementation influences milk fat by its involvement in VLDL synthesis (Sharma and Erdman, 1988; Zanton et al., 2014). S-adenosylmethionine can aid in the formation of choline, carnitine, creatine, and folic acid, while also aiding in providing substrates for milk fat synthesis (Emmanuel and Kenelly, 1984). The synthesis of choline from Met may be one way in which lipid transport is improved by LDLs (Auboiron et al., 1995). These LDLs are capable of transporting lipids throughout the body. Increased LDL transport can result in greater lipid transport making them more readily available to the mammary tissue. On average, the supplementation of RPM sources results in a 1.9 g increase in milk fat for each gram of MP Met supplied (Zanton et al., 2014). Met supplementation by HMTBa and post ruminal DL-Met resulted in an increase in milk fat concentration ($P < 0.05$) while Mepron and Smartamine tend to result in trends for increase in milk fat concentration ($P < 0.08$) (Zanton et al., 2014). In contrast, Ardalan et al., (2021) provided both Smartamine M and HMBTa and observed no effect on milk fat percentage or milk fat yield (3.49 % and 1.60 kg/d). Many of these studies where HMTBa was given were designed around the idea of overcoming milk fat depression induced by feeding increased amounts of concentrate and as a result the differences in experimental objectives may play a role in the differences in improvement Met. Patton, (2020) reported in their meta-analysis that milk fat yield was impacted by RPM product, with Smartamine M having no effect on milk fat yield, while Mepron increased milk fat yield with
supplementation by 11g. Milk fat yield can be improved through RPM supplementation by its involvement in the choline/SAM pathway VLDL transport, with an average increase in milk fat yield of 1.9 g per gram of MP Met supplied.

1.8 Assessing bioavailability

Due to the nature of the encapsulation technology applied to the product, there is some loss in bioavailability to the animal. Specifically, some percent of product is lost due to rumen degradation, and/or some lost due to the coating inhibiting the absorption at the small intestine. Although some loss is unavoidable, the extent of loss can vary substantially among products. The encapsulated products vary in size of the supplements, concentration of methionine or amino acid used and density of said products (Robinson et al., 2011). Bioavailability to ruminants also varies largely depending on the encapsulation technique of these products (Robinson et al., 2011). Encapsulation techniques can vary from type of coating such as lipid or polymer, pH sensitivity and others all which can impact how the molecules are made available to the animal. This has led to the development of procedures to determine the availability of different protected amino acid products. Differing in vitro and in vivo techniques may be used to estimate the availability of these different products for ration formulation.

In vitro techniques involve the simulation of the rumen, abomasum, and intestines using buffered rumen fluid and enzyme solutions to estimate the bioavailability of these products. Ross et al. (2013) developed one of the most widely used in vitro methods utilizing the RUP fraction of feedstuffs paired with a measure of intestinal digestibility to estimate ruminal degradation and intestinal absorption of RPAA. In vitro techniques for
assessing RPAA bioavailability remain one of the best for screening new prototype products due to the ability to quickly determine predicted availability, and for ease of environmental control of these techniques allowing for ease in replicability. Limitations often faced by in vitro methods include difficulty in modeling the complexity associated with the living organism due to challenges faced in modeling a living organism and its processes. Furthermore, this technique eliminates the mechanical stresses and rumination effects that can impact release of product. Due to the limitations, preliminary in vitro findings should be validated through in vivo techniques.

In vivo techniques utilize the living organism to evaluate effects of a treatment, resulting in a more accurate understanding of how a product will be utilized and affect the organism. There have been several in vivo methods developed for measuring bioavailability ranging from the production response approach (Schwab et al, 2001) to plasma free AA dose response, measuring plasma increase in free AA (Rulquin and Kowalczyk, 2003; Graulet et al, 2005; Whitehouse et al, 2017). There are certain constraints associated with each of these methods which should be considered when reviewing their results. A production response approach estimates bioavailability by analyzing the slope of the response (most commonly milk protein concentrations or yield) in response to known concentrations of administered AA treatments (Schwab et al, 2001). Production response techniques require that the ration be formulated in such a way that the animal is deficient in the desired AA, otherwise a linear response will fail to be seen across AA treatments.
Free plasma AAs increase when AAs are absorbed across the lumen of the small intestine and transported throughout the body by plasma. Plasma Met is a strong indicator of bioavailability to animals and is directly influenced by intestinal absorption of Met (Rulquin and Kowalczyk, 2003; Whitehouse et al., 2017). Two methods that use this plasma response to measure bioavailability are the plasma free AA dose response and area under the curve/pulse dose response.

Plasma free AA dose response is a steady state approach, which utilizes the linear relationship between incremental amounts of AA supply and plasma concentration of that free AA to estimate bioavailability. The increases in plasma AA concentration reflect an increase in net absorption of AA, from which a relative bioavailability can be determined (Whitehouse et al, 2017). Rulquin and Kowalczyk (2003) first developed a method to quantitatively assess AA bioavailability based on the plasma AA response, and this method was further refined by Whitehouse et al. (2017). Cows are organized in a Latin square design in which animals receive either abomasally or duodenally infused raw AA, fed RPAA or no supplemental AA. Blood samples are collected and analyzed for free plasma AA. Regression analysis is performed on plasma AA to determine the increase in plasma free AA in response to the infusion or feeding of treatment. The bioavailability of RPAA can then be calculated from the regression assuming infused raw AA is 100% available. It should be noted that when examining bioavailability, considerations should be made regarding diet composition. Diets should be formulated to be in excess of protein, approximately 115%, to ensure experimental accuracy (Rulquin and Kowalczyk, 2003). Rations must be formulated to be sufficient in Met, according to the NRC (2001), to ensure
that Met deficiency does not negatively affect the plasma AA profile. Steady state has been shown to be achieved as soon as day four of infusions (Rulquin and Kowalczyk, 2003), while Whitehouse et al. (2017) identified steady state by day 7. Plasma samples should be collected at several time points throughout the day and for a minimum of 3 consecutive days to obtain a representative sample (Whitehouse et al, 2017).

Area under the curve utilizes a pulse dosage of RPAA into the rumen, abomasum, or duodenum. Serial blood samples are then collected to calculate the area under the curve of the plasma AA response, and this can be repeated to compare several different equimolar AA sources (Schwab et al, 2001; Graulet et al, 2005). Bioavailability can be calculated relative to the abomasally or duodenally infused AA like that of the steady state approach. Using regression analysis on plasma AA to determine the increase in plasma free AA in response to the infusion or feeding of treatment. Bioavailability is then calculated from the regression assuming infused raw AA is 100% available. A limitation of this technique is that it supplies animals with a supra-physiological amount of AA which may reduce rumen degradation and/or overwhelm the capacity for intestinal absorption. The potential for a regulatory response and increased catabolism are of concern amongst pulse doses for plasma techniques (Flemming et al, 2019).

Both plasma free steady state response and pulse dose response share similar disadvantages including difficulty in managing variables, cost, possible ethical concerns depending on type of investigation, as well as difficulty in comparing bio availabilities of products which utilize different delivery mechanisms. Additionally, a challenge in the assessment of encapsulated RPM is these techniques can provide correct relative
differences among products but may result in underestimate of bioavailability (Whitehouse et al, 2017; Fleming et al, 2019).

Smartamine M has been one of the most extensively studied RPM products resulting in its position as a benchmark in RPM research. Smartamine M’s bioavailability was estimated at 80% by Schwab (1996) in a nylon bag study. This result was further reinforced with estimated availability between 75.0 and 97.1% in an estimated digestibility test (NRC, 2001). Rulquin and Kowalczyk, (2003) estimated availability at 75 ± 11% in their study utilizing steady state plasma Met levels in response to a linear calibration curve established from duodenal infused DL Met. Due to numerous repeated results across a number of studies firmly documenting a bioavailability of 80%, Smartamine M secured its status as a benchmark product, with a Met content of 75%, allowing it to be utilized as a reference value modeling response to Met products (Schwab, 1996; Graulet et al, 2005). Rumen protected products, with known and well documented bioavailability and Met percent, can be used in plasma AA in-vivo models to confirm bioavailability based upon plasma Met appearance and rumen disappearance (Schwab, 1996; Rulquin and Kowalczyk, 2003; Whitehouse et al, 2017). Bioavailability of prototype or commercial products can be estimated through implementation of milk protein concentration response from lactational trials and manufacturers’ standards and values. Through comparing experimental products to Smartamine M, one can estimate supplied Met content of similar products by establishing linear response. With a known Met content, the change in milk protein or free plasma Met concentration can be measured and compared with that of Smartamine M to determine supplied bioavailable Met. When assessing for milk protein response, if cows
are not substantially deficient in Met or other AA of interest, a declining apparent bioavailability can be seen for incremental doses.

1.9 Conclusion

The supplementation of cows with MP Met has long been understood to benefit production performance especially when the goal of maximizing efficiency is of interest to meet nutritional demands of high producing animals. Though ruminants can produce a balanced AA profile from MCP given a properly formulated ration, MCP alone can fail to meet the quantities of EAA necessary for the mammary gland of high producing animals. Both RDP and RUP of a diet influences performance and MCP production, however the RUP fraction represents the AA profile which is available to the cow for milk production and milk component yields. A better understanding of MP, ME, and EAA requirements has allowed for better modeling and understanding of how to formulate rations with animal performance and health in mind. Methionine's ability to influence animal performance in high producing animals has long been documented, with improvements to milk protein, milk yield and milk fat. It acts as a fundamental building block to a variety of other key proteins and lipoproteins. Met enhances lipid transport through involvement in a variety of related pathways through donation of its methyl groups for lipid biosynthesis and is involved in transmethylation. Met can mobilize fats and aid in the synthesis of PC and related compounds though the SAM pathway. Phosphatidylcholine and VLDLs can be critical in TG clearance of lipids from the liver, helping to reduce metabolic disorders often seen in transition cows. Successful supplementation of Met has made use of encapsulate technologies including polymer and lipid coatings which can supply bioavailable MP Met.
The supplementation of RPM has resulted in increased milk yield, milk protein, and milk fat. Bioavailability of these products is important to ascertain, especially as technology and modeling continues to evolve. Bioavailability of RPM products can be successfully estimated by establishing a linear relationship between plasma Met levels and duodenally or abomasally infused Met or fed RPM product. Plasma free AAs serve as an excellent indicator of bioavailability, due to their transport across the lumen of the small intestine and movement throughout the body by plasma. An increase in plasma Met levels can indicate bioavailability to animals and is directly influenced by intestinal absorption of Met.
Chapter 2

OBJECTIVES

KESSENT M (RPM-K; Kemin Industries, Des Moines, IA, USA) was developed as a competitive rumen stable product to commercially available RPM products. Experiment 1 examined plasma methionine response and effect on animal performance of RPM-K and commercially available products in Holstein dairy cows to assess the products bioavailability. Cows were provided treatments which were administered at time of feeding three times daily and consisted of a control diet plus 12 g/d of one of three rumen protected Met products, either the newly developed product RPM-K or one of two existing products, RPM-S and RPM-M, with known differences in bioavailability. The effectiveness of these products would be determined by their impact on plasma methionine response to the feeding of specific products.

For Experiment 2, the objective of the experiment was to evaluate performance response of early lactation dairy cows to a newly developed product (RPM-K) to an existing product with high demonstrated bioavailability (RPM-S). Both products protect Met by using a pH sensitive polymer that is resistant to ruminal degradation but releases in the intestines. Due to the similarity in technology, we hypothesized that RPM-K would elicit comparable responses in early lactation cows as RPM-S. Treatments consisted of a control diet deficient in MP Met by 17 g, or control diet plus 14 g/d one of two RPM products (RPM-K or RPM-S). Milk samples were collected on d 13-14 and 18-21 of each
period. Plasma samples were collected at 2 and 6 hours after feeding on d 21 of each period and subjected to free amino acid analysis.
Chapter 3

EVALUATION OF PLASMA AMINO ACID RESPONSE OF A NEWLY DEVELOPED RUMEN PROTECTED METHIONINE PRODUCT TO TWO CURRENTLY MARKETED PRODUCTS IN HOLSTEIN DAIRY COWS

3.1 Introduction:

The ability to influence production parameters, specifically milk protein, is of considerable interest to dairy farmers and nutritionists. Milk protein production is heavily influenced by how efficiently cows utilize MP for protein synthesis and is dictated by essential AA profiles found in this MP. There has been substantial research indicating that Met is one of the first limiting AA for dairy cows fed corn silage and alfalfa silage diets common in the US (Overton et al., 1998; NRC, 2001). Post ruminal infusions of increasing amounts of Met have been demonstrated to improve milk protein production (Pisulewski et al., 1996). Rumen protected methionine (RPM) was developed to supply Met in a form protected from rumen degradation and available for absorption across the small intestine. Supplementing RPM to improve EAA profiles could also allow for the reduction in RUP of diets, reducing the amount of dietary N and subsequent excretion into the environment (NRC, 2001). Bioavailability of rumen protected amino acid (RPAA) products can vary largely, dependent upon encapsulation technique and chemistry, but relative differences can be ascertained though analyzing plasma AA response to the feeding of said products (Rulquin and Kowalcyk, 2003; Whitehouse et al., 2017). Free plasma AA is a strong
indicator of bioavailability to animals and responds linearly to intestinal absorption (Rulquin and Kowalczyk., 2003). When cows are fed isonitrogenous diets, differences in availability of RPM products can be assessed by evaluating differences in plasma free Met (Papas et al., 1984; Overton et al., 1996; Soder and Holden, 1999; Südekum et al, 2004).

The primary objective of this study was to compare the plasma Met levels of cows fed a newly developed RPM product to two existing products with known differences in plasma Met response. We hypothesized that new product would elicit comparable plasma Met responses to that of the more available product. Measurement of free amino acids in plasma samples is costly. Thus, a secondary objective was to determine how theoretical pooling of individual cow samples would affect data interpretation.

3.2 Materials and Methods:

Animals and Treatments: Ten multiparous Holstein cows mid to late lactation were moved to a tie stall facility seven days prior to the start of the experiment for a 1-week adaptation period (November 23, 2018). Cows utilized had a mean DIM of 280 (± 73) at the beginning of the trial. Cows were fed three times daily at approximately 8 am, 4:30 pm, and 12 am, with 33% of their daily feed allotment provided at each feeding. Cows were fed ad-libitum for ~5% orts. Cows had free access to individual waterers throughout the study. Cows were milked twice daily (~4:30 am and 3:30 pm) and were away from their stalls approximately 4:30 – 8 am and 3:30 - 4:30 pm. Body weights measured on two consecutive days at the end of the adaptation period was 755 (± 56) kg.

The experiment was conducted over 4 weeks, with the 1-week adaptation period followed by 3 seven-day periods using a replicated 3×3 Latin square design. During the
adaptation period, cows were fed a ration which had been formulated to contain sufficient levels of all essential amino acids (Table 1). At the end of the adaptation period cows were assigned to blocks by DIM, and randomly assigned to treatment sequences within each block. Nine cows were assigned to 3 complete blocks and the remaining cow was assigned to an incomplete block.

The three experimental treatments consisted of the control diet plus 12g/d of either KESSENT M (RPM-K; Kemin Industries, Des Moines, IA, USA), or Smartamine M (RPM-S; Adisseo Inc., Antony, France), or Mepron (RPM-M; Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany). The RPM-S treatment was used as a positive control because it has been consistently shown to have a high Met bioavailability of approximately 80% (Schwab, 1996; Graulet et al, 2005). The RPM-M treatment was included as a negative control because it has a substantially lower bioavailability when assessed by comparing the plasma free Met response to RPM-S (Blum et al, 1999; Südekum et al, 2004). Prior to each feeding time, ~1 kg TMR was mixed into small tubs with treatments and placed in front of the cows. Cows were given access to the TMR and supplements for 15 minutes. If the mix was not consumed within 15 minutes, the remainder was swept up and placed on top of fresh feed. RPM-K was provided by Kemin Industries Inc., and RPM-S and RPM-M were purchased from Renaissance Nutrition (Roaring Springs, PA).

**Milk and Feed Sampling:** Milk yield was recorded for each cow at time of each milking throughout the study and milk samples were collected during am and pm milking (~4:30 am and 3:30 pm) days 5-7 of each period and submitted to Dairy One (Ithaca, NY).
for NIR analysis of lactose, protein, fat, somatic cell count (SCC) and milk urea nitrogen (MUN) using a MilkoScan System 4000 (Foss) and somatic cell count using a Fossomatic 400 (Foss).

Feed offered and refused was recorded daily. Samples of wet forages were collected 3 times a week during the am feeding and composited by week. Grain mix was sampled once a week. A portion of each feed sample was dried for 48 h at 60 °C in a forced air oven and results were used to correct the ration for dry matter fluctuation. Weekly composite sample were analyzed using wet chemistry methods by Cumberland Valley Analytical Services (Waynesboro, PA) for wet chemistry analysis of DM (105°C for 3 hr for forages; method 930.15, AOAC International, 2000, for grain), neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash (Van Soest, Robertson, & Lewis, 1991), acid detergent fiber (method 973.18, AOAC International, 2000), crude protein (method 990.03, AOAC International, 2000), starch (Hall, 2009), ash (method 942.05, AOAC International, 2000).

Blood Sampling: Blood samples were collected 2 and 6 h after feeding on the last day of the adaptation period from the coccygeal vein. During each experimental period, jugular catheters (ICU Medical, Inc.; Lake Forest, IA) were placed in each cow on day 4. Blood samples were collected on day 5 through 7 of each period at 2, 4, 6, and 8 h after the first feeding. 10 ml of blood was collected at each time point into ethylenediamine tetra-acetic acid (EDTA) coated tubes (Becton Dickinson; Franklin Lakes, NJ). Blood samples were centrifuged at 2,000 x g for 20 min at 4°C after each collection to isolate plasma.
Each plasma sample was aliquoted into two 2 mL cryovials and stored at -80° C. Jugular catheters were removed following the last blood sample taken on day 7 of each period. At the end of the experiment, one set of plasma samples was mailed to the University of Missouri Agriculture Experiment Station Chemical Laboratories for amino acid analysis by cation-exchange chromatography with an amino acid analyzer.

**Statistical Analysis:** For intake and milk yield, mean data from the last three days of each experimental period was used. Mean milk component data from the last three days of each period was weighted by milk yield at each individual milking. Data was evaluated using the GLIMMIX procedure of SAS (SAS Institute Inc., 2011) using a model that included the fixed effects of treatment, period, and block and the random effect of cow.

Plasma free amino acid concentrations were evaluated using the GLIMMIX procedure of SAS using a model that included fixed effects of treatment, period, block, day of sampling and hour of sampling, and all two and three way interactions of treatment, day, and hour. Cow was included as a random effect and mean plasma methionine concentration from the adaptation period was included as a covariate. Hour was included as a repeated measure with the subject of period × day × cow, and a first order autoregressive covariance structure was used.

To assess the theoretical impact of pooling plasma samples on plasma Met results, mean plasma Met concentrations were determined for each cow within a day (mean of the 2, 4, 6, and 8 h samples) and for each cow within a period (mean of the 12 samples collected over the 3 days). Samples theoretically pooled by day were evaluated using the same model
used for the full data set except that hour and interactions with hour were excluded from the model and the repeated measure subject was period × day. The model was further refined to evaluate the samples theoretically pooled by period by removing day, interactions with day, and the repeated statement from the model.

For all models, significance was declared at $P \leq 0.05$, while trends were discussed at $0.05 < P \leq 0.10$. When a fixed effect was observed ($P \leq 0.10$), the pdiff function of SAS was used to differentiate among effect levels.

### 3.3 Results and Discussion:

Ingredient and nutrient composition of the basal ration is presented in Table 1. The ration was formulated to contain 20.5% CP, 29.7% NDF, 17.9% ADF, 22.0% starch, 7.6% ash, and 1.68 Mcal/Kg NE$_L$. The analyzed nutrient composition of the ration (Table 1) was similar to formulated values. Using CNCPS v6.5 (Van Amburgh et al., 2015), the software NDS, and average milk production and composition collected during the experiment, Met and Lys were predicted to be 2.24 and 5.76% of MP, respectively. Predicted required and supplied amounts of Met were 70.5 and 80.6 g/d, respectively, and required and supplied Lys was 187.1 and 207.8 g/d, respectively. All other essential amino acids were similarly predicted to be supplied at levels greater than requirements (data not shown).

*Production response.* There were no effects of treatment on intake, milk yield or milk composition (Table 2). Milk urea nitrogen was elevated at approximately 17 mg/dL and milk protein was high at approximately 3.65% across all treatments. Those factors
combined with the lack of treatment differences in milk protein suggest that we achieved our goal of exceeding dietary amino acid requirements with our ration formulation. The lack of production response to RPM supplementation was a predictable outcome as when diets are formulated to contain sufficient essential AA there is minimal impact of additional supplemented Met on milk yields, milk fat, milk protein, lactose, and SCC (Blum et al., 1999). This result was desirable as it ensured that supplemented RPM product should be detectable in the blood stream versus diverted for productive use.

*Plasma amino acids.* Plasma methionine was affected by treatment ($P < 0.001$) and hour ($P = 0.001$; Table 3). Interactions of treatment × hour, treatment × day, hour × day, and treatment × hour × day were not found ($P > 0.16$). The absence of a day effect ($P = 0.54$) indicated that the plasma response had stabilized by the first day of blood sampling on day 5 of each period. There were no differences between RPM-S and RPM-K (32.7 vs 33.0 µM; $P = 0.75$), and both were greater than RPM-M (30.1 µM; $P < 0.001$). The time effect was due to plasma Met being lowest at 4 h (30.2 µM) as compared to levels at 2, 6 and 8 h (31.9-33.0 µM) post feeding.

The interaction of treatment × hour did not affect any amino acid except for a tendency that was observed for Phe ($P = 0.08$). This was due to a lower concentration of Phe for RPM-K at 4 h (58.4 µM) compared to RPM-K at 2 h, RPM-M at 4 h, and RPM-S at 6 h (63.6, 64.0, and 64.0 µM, respectively). In addition to Met, treatment also affected Cys ($P = 0.009$) and tended to affect Ala ($P = 0.06$), Leu ($P = 0.07$), and Val ($P = 0.053$). Cysteine followed the same pattern as Met, with no difference between RPM-S and RPM-
K ($P = 0.42$), but both were greater than RPM-M ($P \leq 0.03$). The tendencies for effect of treatment on Ala, Leu, and Val were due to Ala being greater in RPM-K than RPM-S ($P = 0.02$), Leu being greater in RPM-M than RPM-S ($P = 0.02$), and Val being lower in RPM-S than RPM-M ($P = 0.02$) or RPM-K ($P = 0.09$). An effect of hour of sampling occurred or tended to occur for the majority of plasma amino acids.

In general, greater plasma amino acid concentrations were observed at 6 and 8 h compared to 2 and 4 h (data not shown). Specifically, time effects for Arg and Asn were the same as observed for Met, with lower plasma levels at 4 h compared to all other times. For Leu, Lys, Thr, and Tyr, plasma concentrations at 4 h were less than at 6 and 8 h, but 2 h did not differ from any other time. For Ala and Cys, plasma concentrations at 2 h and 4 h were both lower than 6 h and 8 h. For Gly and His, plasma concentrations were lowest at 4 h and greatest at 8 h. Intermediate levels were found at 2 and 6 h which differed from both 4 and 8 h. For Ser, 4 h was lower than all other times, 2 h was lower than 8 h, and 6 h did not differ from 2 or 8 h. For Trp, there was no difference between 2 h and 4 h which were both lower than 6 h, and 8 h was greater than all other times. Differences in this pattern were observed for Asp, Gln, Glu, Ile, and Pro. The most dramatic difference was noted for Glu, where plasma concentrations at 4 h were greater than at 2 h or 8 h, and 6 h was also greater than 8 h. For Gln and Pro, concentrations at 4 h were lower than at 6 h, and 6 h was lower than both 2 and 8 h which did not differ from one another. For Asp, lower values were observed at 2 h than at 6 or 8 h, and 4 h did not differ from any other time. For Ile, 6 h was greater than 2 or 4 h, and 8 h did not differ from any other time.
The RPM-S and RPM-M treatments were included as positive and negative controls, respectively, due to their demonstrated differences in plasma Met response (Blum et al., 1999; Südekum et al., 2004). Differences in plasma Met concentrations between RPM-M and RPM-S most likely reflect differences in degrees of protection of Met against ruminal degradation or availability for intestinal absorption. In an in vitro study using rumen inoculum from sheep, Mbanzamihigo et al. (1997) demonstrated that RPM-M had reduced protection from rumen degradation than RPM-S. Intestinal availability of absorption may also be lower for RPM-M than RPM-S, as intestinal disappearance of RPM-M assessed using the mobile bag technique has ranged from 47.8 to 62.5% (Berthiaume et al., 2000; Overton et al., 1996). If the RPM-M product is not able to release properly at the intestines that would serve as one possible reason for the reduced plasma response observed with its treatment.

Our results were consistent with studies which demonstrated that changes in plasma AA concentrations can be used to differentiate availability of different rumen protected amino acid products (Rulquin and Kowalczyk., 2003; Ordway et al., 2009; Whitehouse et al., 2017). Südekum et al. (2004) reported increases in plasma Met in steers fed 50 g/d of D,L Met from RPM-M and RPM-S; however only RPM-S produced a significant increase in plasma Met levels above basal plasma values (463.64 vs. 23.0 μM, respectively) while the increase for RPM-M was not significantly above baseline (38.8 vs. 24.5 μM). Similarly, Blum et al. (1999) reported that a 50 g/d dose of Met from RPM-S fed to lactating dairy cows caused a nine-fold increase in free plasma methionine compared to baseline (144.8 vs. 16.6 μM, respectively) while a two-fold increase was observed when feeding 50 g/d of
Met from RPM-M (29.3 vs. 15.7 µM, respectively). Although our study was not designed to evaluate changes from baseline, plasma samples collected during the adaptation period had a mean plasma free Met concentration of 25.7 µM. Thus, supplementation with RPM-S and RPM-M resulted in numeric increases of 27% and 17%, respectively, relative to the adaptation period. Due to the lower level of supplementation in the present experiment (9.0 g/d DL Met for RPM-S and 10.2 g/d for RPM-M), the magnitude of the response was less than that observed by Blum et al. (1999) and Südekum et al. (2004), but still resulted in similar ranking of the products. These results support their use as positive and negative controls in the present experiment, respectively.

RPM-M is a commercial product which utilizes a rumen stable delivery system of 85% DL-Met with small amounts of starch, fat, ash and a coating of ethyl cellulose, and stearic acid film (Schwab, 1996). The resultant slow-release lipid-coated product contains a high payload of DL-Met in a pelleted product resistant to rumen degradation and available for release at the intestines (Waterman et al, 2012). RPM-S utilizes a lipid/pH-sensitive copolymer to protect its 75% DL-Met core. RPM-S releases DL-Met in response to the lowering of pH, such as that found in the abomasum, and uses an ethyl cellulose coating with an additional poly (2-vinylpyridine-co-styrene) polymer added to the stearic acid, giving its coating the unique pH sensitivity and bioavailability of 80% (Graulet et al, 2005). RPM-K is a more recently designed product which utilizes a similar pH sensitive copolymer coating around a DL-Met core making it pH sensitive like RPM-S. RPM-K makes use of a vinyl pyridine/styrene copolymer which resists degradation by the rumen and begins releasing Met at the acidic environment found in the abomasum. Differences
between RPM-S and RPM-M in plasma methionine response may be due to protective technology of these products. Both RPM-S and RPM-K utilize a pH sensitive coating for release of D, L Met, which may help to explain the similar plasma free Met response, while RPM-M relied on a lipid and stearic acid film, which is time dependent rather than pH reliant (Richard et al., 2012). Räisänen et al. (2020) observed a greater increase in plasma methionine levels in animals given pH sensitive (2-vinylpyridine-co-styrene and other polymers) treatments as compared to lipid coated (hydrogenated vegetable fats and oils) treatments relative to baseline. Products coated in pH sensitive coatings increased plasma Met concentration to 44.9 µM from a base level of 19.6 µM, while their lipid coated Met products increased plasma Met to approximately 25.5 µM when provided to mid lactation dairy cows (Räisänen et al., 2020).

An effect of time on plasma Met is not unexpected as the RPM products were pulse dosed every 8 h, and differences over time may be related to kinetics of passage and absorption of the products. However, similar time effects were observed for other plasma amino acids, suggesting this effect may have been driven by timing of TMR allocation. Effect of meal pattern on timing of plasma amino acid appearance has been demonstrated in pigs. When examining the portal net appearance of amino acids in pigs fed equal amounts three times daily, Reverter et al., (2000) reported that the free EAA in the hepatic portal vein reached peak concentrations 30 to 90 minutes after the initial feeding, while NEAA reached maximum levels between 90 and 120 minutes followed by a decline in plasma concentrations when provided various diets. Lathica et al., (2018) in a similar study examining net portal appearance of EAA and NEAA in Iberian pigs reported rapid
appearance of both EAA and NEAA in the portal and arterial concentrations within 30 minutes after the initial feeding of diets with a gentle decrease in AA concentrations, thereafter, beginning around 2.5 hours later. It appeared that for monogastric plasma amino acid levels for both EAA and NEAA could be expected to begin falling 2-3 hours after initial feeding at a gradual rate due to hepatic metabolism until the return to basal levels (Reverter et al., 2000; Lathica et al., 2018). However, similar responses have not previously been demonstrated in ruminants. Whitt et al., (1996) observed that portal AA flux was not impacted by time of feeding in cattle fed twice daily. A similar observation was made by Rémond et al., (2002) where no effect of time of feeding was observed in sheep when fed fresh forage twice daily. A lack of a response in ruminants is believed to be due to rumen emptying which was suggested to smooth out variations in digesta flow to the duodenum (Whitt et al., 1996). Although ruminal retention impacts the time frame between feed consumed and appearance of AA in the blood stream when comparing monogastrics to ruminants, our data supports a temporal response to feeding on the appearance of AA in the blood similar to that observed in monogastric.

In the present study, the majority of plasma amino acids had greater concentrations at 6 and 8 h compared to 2 and 4 h. Those 6 and 8 h points may be comparable to the 0.5 to 2.5 h elevated levels observed in pigs. Reverter et al. (2000) and Lathica et al. (2018), reported Arg, Asn, Cys, Leu, Lys, His, Met, Thr, and Tyr to be among the AAs which appeared to be especially sensitive to meals which are among the AAs which we observed to have their lowest levels at 2 and 4 h post feeding and highest 6 and 8 h in our study. Asp, Gln, Glu, and Pro were among the AA not reported to be sensitive to time of feeding,
according to Reverter et al. (2000) and Lathica et al., (2018). Interestingly, those were among the amino acids in the present study that differed in their response to time following feeding. An exception was Ile, which had been reported to be meal sensitive by Reverter et al. (2000) and Lathica et al. (2018) but was among the meal sensitive AAs that differed from the others behavior in our study.

Though our data suggests that the time effect on plasma Met was most likely driven by the time of feeding vs. the time of product dosing, multiple previous studies have indicated a relationship between time of RPM treatments and plasma Met appearance. Bach and Stern (2000) esophageal dosed different RPM products using a bolus gun. Plasma Met peaked by 12 h for more slowly degradable products and between 6 and 12 h for moderately degradable RPM. Koenig and Rode (2001) fed cows 30 g or 60 g of slowly degradable RPM-M and observed peak plasma concentration 12 h after dosing. Two studies evaluating RPM-S also observed peak plasma Met at 12 h following feeding or ruminal dosing (Graulet et al., 2005; Toledo et al., 2017). Following the single pulse dose, plasma Met returned to basal levels after 24 to 36 h (Bach and Stern, 2000; Graulet et al., 2005; Toledo et al., 2017). In the present study, treatments were administered every 8 h, and 12 h following product dosing (when others observed peak response to a pulse dose of RPM) equates to our 4 h sampling point. Because we observed the lowest plasma Met concentration at 4 h, this again suggests that the temporal effect was due to timing of TMR allocation rather than timing of Met dosing.
**Conclusions:** Orally administered RPM products provided to mid to late lactation dairy cows fed diets sufficient in dietary Met levels can be differentiated between through post dosage plasma Met analysis. Both RPM-K and RPM-S displayed greater plasma Met than the RPM-M product, suggesting that the bioavailability of both RPM-K and RPM-S are comparable and greater than that of RPM-M. There was a strong impact of time of sampling on plasma Met and most of the other amino acids, demonstrating the importance of collecting multiple samples post-feeding when assessing plasma amino acid response. Pooling individual cow plasma samples by day would have likely resulted in the same differentiation of treatments but pooling by period would have masked some of these differences.
Chapter 4

EVALUATION OF ANIMAL PERFORMANCE RESPONSE TO TWO RUMEN PROTECTED METHIONINE PRODUCTS IN EARLY LACTATION HOLSTEIN COWS

4.1 Introduction:

Limiting EAA prove to be of great importance for improving milk protein synthesis in dairy cows, especially in high producing animals, and resulting in relatively high requirements for dietary MP. It is possible to have diets sufficient in MP but deficient in specific EAA, which can hamper milk production (Colmenero and Broderick, 2006). There has been substantial research indicating that Methionine (Met) is one of the first limiting AA for dairy cows fed diets common in the US (Overton et al., 1998; NRC, 2001). Met supplementation has been commonly observed to increase milk protein percent and yield in diets predominantly consisting of corn silage (Schwab et al, 2001; Rulquin et al, 2006; Socha et al., 2008). Additional benefits of increased Met in the diet include increased milk yield and fat content (Overton et al., 1998; Blum et al, 1999), as well as gains in milk yield during early lactation (Polan et al., 1991). Rumen protected methionine was developed to increase supply of post-ruminal methionine to the animal in such a way it is protected from rumen degradation and allows for absorption across the small intestine. Increased post-ruminal supplies of Met have been shown to increase protein synthesis in the mammary
glands (Pisulewski et al., 1996; Rulquin et al., 2006). Other benefits of supplemental RPM in diets to improve EAA profiles may allow for the reduction in RUP of diets, reducing the amount of dietary N and subsequent reduction in its excretion into the environment (St-Pierre and Thraen, 1999; NRC, 2001). The objective of this experiment was to evaluate performance response of early lactation dairy cows to a newly developed product (RPM-K) to an existing product with high demonstrated bioavailability (RPM-S). We had shown that RPM-K was available to cows in experiment 1, and as a result wanted to test its performance against a commercially successful product. Both products protect Met by using a pH sensitive polymer that is resistant to ruminal degradation but releases in the intestines. Due to the similarity in technology, we hypothesized that RPM-K would elicit comparable responses in early lactation cows as RPM-S.

4.2 Materials and Methods:

This study was approved by the University of Delaware, Animal Care and Use Committee and was conducted from February 2019 through April 2019.

**Animals and Treatments:** The experiment utilized 30 Holstein cows, 24 were multiparous and 6 were primiparous with a mean DIM at the start of period 1 of 95 (± 20) and 71 (± 3), respectively. Cows were moved to a facility with a Calan Broadbent Feeding System (American Calan Inc., Northwood, NH) and trained over a 2-week period prior to the start of the experiment. After the training period cows were transitioned to a control ration (Table 4) for a 14-day adaptation period. The control ration was formulated to contain methionine at 0.87 g/Mcal ME and 2.21% of MP and to
be deficient in MP methionine by approximately 17 g/d. Lysine was formulated at 3.02 g/Mcal ME and 7.65% MP. At the end of the adaptation period cows were blocked by parity, DIM, and milk yield, and assigned to replicated 3×3 Latin squares. Each treatment period lasted 3 weeks.

The three experimental treatments consisted of a control diet deficient in MP methionine by approximately 17 g/d (CON) and control diet plus 14 g/d of either KESSENT M (RPM-K; Kemin Industries, Des Moines, IA, USA) or Smartamine M (RPM-S; Adisseo Inc., Antony, France). Products: RPM-K was provided by Kemin Industries Inc and RPM-S was purchased from Renaissance Nutrition (Roaring Springs, PA).

During the adaptation and experimental periods, cows were fed ad-libitum once daily (~8:00 am), allowing for 5-10% orts. During the experimental period, supplements (RPM-K or RPM-S) were top dressed in equal amounts twice daily, at the time of feeding (~8 am) and prior to return from the afternoon milking (~3:30 pm).

Cows were milked twice daily (~4:30 am and ~3:30 pm). Body weight was measured on three consecutive days at the end of the adaptation period and at the end of each experimental period.

Milk and Feed sampling: Milk yield was recorded at each milking throughout the study. Samples of milk were collected at both the am and pm milking during the last 4 days of the adaptation period. During each experimental period, milk samples were collected on days 6 and 7 of week 2 and days 4 -7 of week 3. Milk samples were submitted to Dairy
One (Ithaca, NY) for NIR analysis of lactose, protein, fat, SCC and MUN using a MilkoScan System 4000 (Foss) and somatic cell count using a Fossomatic 400 (Foss).

Feed offered and refused was recorded daily. Samples of wet forages and TMR were collected 3 times a week during the am feeding and composited by week. Grain mix was sampled once a week. A portion of each feed sample was dried for 48 h at 60 °C in a forced air oven and results were used to correct the ration for dry matter fluctuation. Weekly composite sample were analyzed using wet chemistry methods by Cumberland Valley Analytical Services (Waynesboro, PA) for DM (105°C for 3 hr for forages; method 930.15, AOAC International, 2000, for grain), neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash (Van Soest, Robertson, & Lewis, 1991), acid detergent fiber (method 973.18, AOAC International, 2000), crude protein (method 990.03, AOAC International, 2000), starch (Hall, 2009), and ash (method 942.05, AOAC International, 2000).

**Blood Sampling:** Plasma samples were collected at 2 and 6 hours after feeding on the last day of the adaptation period and each experimental period. 10 ml of blood was collected at each time point into EDTA-coated tubes (Becton Dickinson; Franklin Lakes, NJ). Blood samples were centrifuged at 2,000 x g for 20 min at 4°C after each collection to isolate plasma. Plasma was stored at -80°C until mailed to the University of Missouri Agriculture Experiment Station Chemical Laboratories for amino acid analysis by cation-exchange chromatography with an amino acid analyzer.
Statistical Analysis:

**Milk production and composition, intake, and body weight**

*Mean calculations.* For the adaptation period, mean intake and milk yield were calculated for the last 7 days of that period. Milk composition was determined as the means of the samples collected during the last 4 days of the adaptation period weighted by milk yield at each individual milking. For weeks 2 and 3 of each period, weekly means of intake and milk yield were calculated. Weighted means of milk composition data were also determined (weighted means of 4 samples collected over days 6-7 for week 2 and weighted means of 8 samples collected over days 4-7 for week 3).

*Removal of outliers data criteria.* One primiparous cow developed clinical mastitis at the start of the second period and was excluded from analysis. Three additional cows (2 multiparous; and 1 primiparous) were excluded from analysis due to abnormality in milk or intake data. Specifically, one multiparous cow experienced a 20% drop in milk yield during period 1, did not recover in milk yield, and then underwent a 50% drop in both milk yield and intake during period 3. The second multiparous cow underwent a 50% decrease in milk yield during period 2 that was sustained for 7 days before steadily increasing to approximately 90% of prior milk yield. That cow also had an 83% decrease in intake on the day corresponding to the lowest milk yield. Intake steadily increased over a 10-day
period to approximately 90% of prior intake. The primiparous cow that was dropped from analysis had a 22.1% coefficient of variation in daily intake as well as variable milk yield.

Milk production, milk composition, and intake data were evaluated using the MIXED procedure of SAS using a model containing fixed effects of treatment, period, week, parity, block, and the interaction of treatment by week and the random effect of cow. Week was included as a repeated measure with the subject of cow × treatment, and a first order autoregressive covariance structure was used. Data collected during the adaptation period were included as covariates. Responses to treatment were determined using two non-orthogonal contrasts. The RPM contrast compared CON to RPM-S and RPM-K, and the Source contrast compared RPM-S to RPM-K.

Bodyweight was evaluated using the same model except that week and the interaction of treatment by week were removed from the model. Plasma methionine data were evaluated using the same model used to evaluate milk and intake data, except that the repeated term of week was replaced by hour of blood sampling. Mean plasma methionine percentage from the 2 samples collected during the adaptation period were included as covariates.

The interaction of treatment by parity was initially included in all models but was never significant ($P > 0.25$) and was removed from the final models. For all models, significance was declared at $P \leq 0.05$, while trends were discussed at $0.05 < P \leq 0.10$.

*Evaluation of model.*
Week. Milk samples were collected during both weeks 2 and 3 of each period. This was done because we previously observed milk component responses to RPM supplementation within 2-weeks, but period lengths were 3 weeks for this experiment. Data were initially analyzed using only the 3-week data or combined data from both week 2 and 3. Predicted LS means were similar for both models so results from both weeks were included in the final model.

4.3 Results and Discussion:

Ration. Analyzed nutrient composition of the TMR was within the formulated values (Table 4). Formulated CP was 15.0% and analyzed CP in the TMR samples was 15.4%. Because TMR samples are also subject to sampling errors due to the difficulty of collecting a fully representative sample, TMR nutrient content was also calculated based on the sum of nutrient content of individual ingredients. Using that method, TMR CP content was nearly identical to formulated at 15.1%. NDF was formulated at 30.4%, but analyzed content was slightly lower at 29.2% (analyzed TMR samples) or 29.4% (calculated from individual diet components). Starch was formulated at 25.5%, but analyzed content was higher at 27.5% (analyzed TMR samples) or 27.1% (calculated from individual diet components).

Production response. Production responses are presented in Table 5. Dry matter intake was affected by Source ($P = 0.02$). This was due to lower DMI for cows on the RPM-K treatment than RPM-S (26.2 vs. 26.6 kg/d). Treatment did not affect yields of milk, milk protein, energy corrected milk, energy corrected milk divided by DMI, SCC, MUN.
or bodyweight. The RPM treatments collectively increased milk fat percentage ($P = 0.02$) and tended to increase milk fat yield ($P = 0.07$) relative to CON, but there was no difference between the two RPM sources ($P \geq 0.47$). Milk protein percentage was affected by RPM ($P = 0.04$) with both treatments increasing protein percentage over CON (3.25 %) but was not different between cows on the RPM-K and RPM-S (3.28 %, $P = 0.98$) treatments. Lactose percentage was reduced by RPM treatments compared to the CON ($P = 0.04$) but were not different from one another ($P = 0.78$).

**Plasma amino acid concentrations.** The plasma amino acid responses are presented in table 6. Plasma methionine concentration was increased by RPM (45.2 μM for both treatments) compared to CON (31.6 μM, $P < 0.0001$), with no difference between RPM-K and RPM-S. The RPM did not affect any other amino acids except for Cys ($P = 0.02$) and a tendency for an effect on Gly ($P = 0.06$). Plasma Cys concentrations were elevated in cows provided RPM-K (16.2 μM) and RPM-S (15.7 μM) treatments as compared to the control (15.1 μM). The plasma concentration of Gly tended to be reduced in cows fed RPM-K (291.8 μM) and RPM-S (289.0 μM) treatments compared to the control (305.7 μM). There was no effect of Source on any amino acids.

Overall, our results are consistent with studies which demonstrated changes to animal performance when supplemented with RPM. That being said, RPM supplementation’s effect on milk yield remains somewhat inconsistent, varying between experimental design and encapsulation technique. Effect of RPM on milk fat remains unclear as there is not always an increase, with studies observing increases in percentage,
yield, or no effect at all. Differences observed in studies may be in part due to encapsulation technology of Met products or diet composition (Yang et al 2010; Zhou et al, 2016). Ordway et al., (2009) reported that milk yield was unaffected when diets were supplemented with 20 g/d of RPM. Lara et al (2006) reported improved milk production \( (P < 0.01) \) with RPM supplementation with quadratic gains \( (P < 0.05) \) to milk yield with a maximum yield at 16.2 g/d of 80% DL-Met from RPM sources. Overton et al (1996) reported increased yields of 3.5% fat corrected milk after supplementation with 20 g/d of an RPM product containing 85% DL-Met when given within the first 126 days of lactation.

We had not expected an effect of treatment on milk yield in our study due to length of experimental periods and the Latin square design, which did not allow for measurement of longer-term effects on supplementation on performance. However, the Latin square design was utilized to increase the likelihood of observing effects of rumen protected methionine supplementation on milk components, and those effects were observed.

The observed gains in milk protein percentage with RPM supplementation were consistent with the literature. Again, supplementation with RPM-K and RPM-S resulted in milk protein percentage of 3.28%, both seeing increases over the control treatment at 3.25%. This observed increase in milk protein percentage is one of the more commonly observed responses when RPM is supplemented in a diet. Socha et al (2008), observed a linear increase in milk protein percentage when supplementing mid-lactation cows with increasing amounts of duodenal infused Met, ranging from 0 - 20 g/d, resulting in milk protein percent increase from 3.22% at 0 g/d to 3.32% at 20 g/d DL Met. In their meta-analysis, Zanton et al. (2014) reported that two of the most supplemented RPM products
resulted in similar increases in milk protein percent (0.07 percentage units) to that of post-ruminally infused DL-Met (0.08 percentage units). Lara et al (2006) reported there was a linear increase in milk protein percentage from 3.35% in the basal diet to 3.52% in response to supplementation of 0, 8, 16, and 24 g/d of an RPM product. Previous studies that have evaluated RPM-S have similarly noted increases in milk protein percentage. Armentano et al (1997) reported linear increases in milk protein percentage from 2.89 to 3.01% when supplementing 5.25, 10.5, and 11.5 g/d DL-Met from Smartamine M. A linear response was reported by Ardalan et al (2021) with protein percentage increasing from 2.77% to 2.87% when cows were supplemented with 0, 7.5, and 15 g/d RPM Smartamine M. Ordway et al (2009) reported improved milk protein response to two RPM products (Smartamine M, 75% DL-Met; MetaSmart, 70%), producing 2.87% and 2.81% milk protein respectively compared to the control of 2.72%.

As mentioned above, milk fat percentage was increased by RPM treatments when compared to the control ($P = 0.02$) and milk fat yield tended to be affected by RPM ($P = 0.07$). Two possible routes by which Met is affecting milk fat is through its influence on de novo synthesis in the mammary gland or mammary uptake of circulating long chain FA (Neville and Picciano, 1997). Met’s involvement in the transference of methyl groups in the form of S-adenosylmethionine, may be the method by which RPM supplementation influences milk fat by assisting in VLDL synthesis and export, consequently increasing availability of circulating fatty acids (Sharma and Erdman, 1988: Zanton et al, 2014). Specifically, S-adenosylmethionine can aid in the formation of choline, phosphatidylcholine, carnitine, creatine and can improve VLDL transport and increase
substrates for milk fat synthesis (Emmanuel and Kenelly, 1984; Auboiron et al., 1995). On average, the supplementation of RPM sources results in a 1.9 g increase in milk fat for each gram of MP Met supplied (Zanton et al., 2014). Patton (2020) reported in their meta-analysis that milk fat yield was impacted by RPM product, with Smartamine M having no effect on milk fat yield, while Mepron increased milk fat yield with supplementation by 11 g.

Lactose percentage was reduced for RPM when compared with the CON ($P = 0.04$). This appears to be in line with the literature, with similar observations being made for cows provided RPM compared to control diets. Junior et al., (2021) reported a decrease in milk lactose percentage for cows supplied with 23 g/d of Smartamine M versus a control (4.54% vs. 4.61%, $P = 0.03$). Similarly, Lee et al., (2019) reported a trend for decrease in lactose percentage in cattle supplemented with rumen-protected Lys (10 g/d) and Met (4 g/d) versus their control in dairy cows (4.90 vs. 4.93%, $P = 0.06$). Typically, differences in milk lactose percent associated with increased milk yield has been reported with RPM-S, but in our case, treatment did not affect milk yield. Met can act as a glucogenic amino acid. Though most of the glucose necessary for lactose synthesis is achieved from gluconeogenesis, Met and other amino acids are not prioritized for glucose production, and therefore the quantitative contribution in the form of glucogenic carbon is small (Aschenbach et al., 2010; Larsen and Kristensen., 2013). There are additional metabolic priorities that Met possesses over gluconeogenesis and lactose synthesis, such as protein synthesis, DNA methylation, SAM dependent trans-methylation reactions, formation of polyamines, synthesis of phosphatidylcholine, and the synthesis of the NEAA cysteine
Perhaps the increased Met draws the glucose necessary for lactose synthesis away from the mammary gland in support of these other Met related pathways, resulting in a reduced lactose percentage as compared with the CON treatment due to this partitioning of energy requirements.

**Plasma methionine.** The RPM treatments increased plasma free Met by approximately 43% relative to CON, with no difference between the two RPM sources. Analyzed N content of the KESSENT M and Smartamine M was 7.28 and 7.59%, respectively, equating to 77.6 and 80.8% methionine respectively. With this data, bioavailability of RPM-K can be calculated relative to known 80% bioavailability of RPM-S (Schwab, 1995) as:

$$\text{RPM-K bioavailability} = \frac{\text{plasma Met with RPM-K} - \text{plasma Met for Control}}{\text{plasma Met with RPM-S} - \text{plasma Met for Control}} \times \frac{80.8\% \text{ Met in RPM-S}}{77.6\% \text{ Met in RPM-K}} \times 80\% \text{ bioavailability of RPM-S}$$

Using the formula, bioavailability of RPM-K is calculated as 83% and roughly equivalent to that of RPM-S.
Chapter 5

CONCLUSIONS

Modern dairies strive to improve feed efficiency and animal performance to improve profitability and reduce cost. RPM products when supplemented in a cow’s diet are one such outlet which may be pursued to increase animal performance and efficiency while potentially reducing cost. In experiment 1, the bioavailability of RPM-K was evaluated and compared against two commercial products. Bioavailability of RPM-K was found to be similar to RPM-S and greater than RPM-M products. Similar results would likely have been obtained had plasma samples been pooled by day for each cow in each period, but not if plasma samples had been pooled by period for each cow.

In experiment 2, the effect of two RPMs on the performance of early lactation dairy cows was evaluated. Supplementing early lactation dairy cows consuming a diet deficient in methionine with RPM products increased milk protein percent and milk fat percent and tended to increase milk fat yield. Both RPM-K and RPM-S caused similar increases in milk fat and protein percentages relative to the CON. Plasma methionine concentration was similar for both treatments suggesting similar effectiveness between products. Both RPM products can be expected to improve milk fat percent and milk protein percent in diets deficient in methionine.
### Table 1. Ingredient composition and analyzed nutrient content of the experimental ration Experiment 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% DM</th>
<th>±   SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>38.60</td>
<td></td>
</tr>
<tr>
<td>Alfalfa silage</td>
<td>13.74</td>
<td></td>
</tr>
<tr>
<td>Ground corn grain</td>
<td>17.22</td>
<td></td>
</tr>
<tr>
<td>Canola meal</td>
<td>15.23</td>
<td></td>
</tr>
<tr>
<td>Expelled soybean meal</td>
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<td></td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>5.35</td>
<td></td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
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<td></td>
</tr>
<tr>
<td>Rumen bypass fat&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Trace mineral and vitamin mix&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Potassium carbonate&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Sugar by-product&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Vegetable fat&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Biotin&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Live yeast&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td><strong>DM, %, ± SD</strong></td>
<td>49.5</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>% DM ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>20.9</td>
</tr>
<tr>
<td>NDF</td>
<td>28.3</td>
</tr>
<tr>
<td>ADF</td>
<td>18.0</td>
</tr>
<tr>
<td>Starch</td>
<td>22.6</td>
</tr>
<tr>
<td>Ash</td>
<td>8.4</td>
</tr>
<tr>
<td><strong>NE&lt;sub&gt;L&lt;/sub&gt;&lt;sup&gt;8&lt;/sup&gt;</strong></td>
<td>1.66</td>
</tr>
</tbody>
</table>

<sup>1</sup> MEGALAC (Church & Dwight Co., Inc, Princeton, NJ).
<sup>2</sup> Contained 5.8% calcium, 34.4% magnesium, 7.3% sulfur, 4.5% potassium, 52 mg/kg Fe, 7,093 mg/kg Zn, 1,223 mg/kg Cu, 5,303 mg/kg Mn, 65 mg/kg Se, 141 mg/kg Co, 191 mg/kg I, 882 KIU/kg Vitamin A, 220 KIU/kg Vitamin D, and 5,292 IU/kg Vitamin E.
<sup>3</sup> DCAD Plus (Church & Dwight Co., Inc, Princeton, NJ).
<sup>4</sup> Contained 92.3% sucrose.
<sup>5</sup> Palmit 80 (Global Agri-trade Corporation, Rancho Dominguez, CA).
<sup>6</sup> Microvit H Promit Biotin 2% (Addiseo, Antony, France).
<sup>7</sup> Levucell SC (Lallemand Animal Nutrition, Milwaukee, WI)
<sup>8</sup> Calculated using NRC (2001).
Table 2. Effect of treatment on intake, milk production and milk composition, for all cows which were provided either control diets plus 12g/d of either KESSENT M\(^1\) (RPM-K), Smartamine M\(^2\) (RPM-S), or Mepron\(^3\) (RPM-M).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RPM-S</th>
<th>RPM-K</th>
<th>RPM-M</th>
<th>SEM</th>
<th>P values Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>27.6</td>
<td>27.8</td>
<td>27.5</td>
<td>1.0</td>
<td>0.82</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>31.5</td>
<td>32.4</td>
<td>30.8</td>
<td>3.9</td>
<td>0.13</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.07</td>
<td>4.02</td>
<td>4.18</td>
<td>0.19</td>
<td>0.29</td>
</tr>
<tr>
<td>Fat, kg/d</td>
<td>1.24</td>
<td>1.26</td>
<td>1.26</td>
<td>0.12</td>
<td>0.93</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.66</td>
<td>3.63</td>
<td>3.69</td>
<td>0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>Protein, kg/d</td>
<td>1.15</td>
<td>1.17</td>
<td>1.13</td>
<td>0.14</td>
<td>0.27</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.51</td>
<td>4.49</td>
<td>4.49</td>
<td>0.04</td>
<td>0.80</td>
</tr>
<tr>
<td>MUN, mg/dL</td>
<td>16.6</td>
<td>17.0</td>
<td>17.2</td>
<td>0.7</td>
<td>0.43</td>
</tr>
<tr>
<td>SCS</td>
<td>4.09</td>
<td>4.23</td>
<td>4.12</td>
<td>0.73</td>
<td>0.93</td>
</tr>
</tbody>
</table>

\(^1\)KESSENT M (Kemin Industries Inc., Des Moines, IA).
\(^2\)Smartamine M (Adisseo USA Inc., Alpharetta, GA).
\(^3\)Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany).
Table 3. Plasma free amino acid concentrations (μM) for cows provided either control diet plus 12g/d of either KESSENT M\(^1\) (RPM-K), Smartamine M\(^2\) (RPM-S), or Mepron\(^3\) (RPM-M).

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>RPM-S</th>
<th>RPM-K</th>
<th>RPM-M</th>
<th>SEM</th>
<th>Treatment</th>
<th>Hour</th>
<th>Treatment (t \times) Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>236</td>
<td>249</td>
<td>242</td>
<td>5</td>
<td>0.06</td>
<td>0.001</td>
<td>0.38</td>
</tr>
<tr>
<td>Arginine</td>
<td>78.6</td>
<td>79.6</td>
<td>77.8</td>
<td>1.7</td>
<td>0.67</td>
<td>0.004</td>
<td>0.20</td>
</tr>
<tr>
<td>Asparagine</td>
<td>55.9</td>
<td>57.4</td>
<td>56.7</td>
<td>1.9</td>
<td>0.64</td>
<td>0.001</td>
<td>0.26</td>
</tr>
<tr>
<td>Aspartate</td>
<td>4.07</td>
<td>4.11</td>
<td>4.15</td>
<td>0.19</td>
<td>0.89</td>
<td>0.008</td>
<td>0.55</td>
</tr>
<tr>
<td>Cysteine</td>
<td>18.3(^A)</td>
<td>18.5(^A)</td>
<td>17.5(^B)</td>
<td>0.5</td>
<td>0.009</td>
<td>0.001</td>
<td>0.76</td>
</tr>
<tr>
<td>Glutamate</td>
<td>39.3</td>
<td>38.1</td>
<td>38.4</td>
<td>1.1</td>
<td>0.18</td>
<td>0.001</td>
<td>0.40</td>
</tr>
<tr>
<td>Glutamine</td>
<td>295</td>
<td>299</td>
<td>298</td>
<td>7</td>
<td>0.78</td>
<td>0.001</td>
<td>0.53</td>
</tr>
<tr>
<td>Glycine</td>
<td>239</td>
<td>245</td>
<td>238</td>
<td>5</td>
<td>0.52</td>
<td>0.001</td>
<td>0.66</td>
</tr>
<tr>
<td>Histidine</td>
<td>69.1</td>
<td>70.2</td>
<td>70.2</td>
<td>1.8</td>
<td>0.50</td>
<td>0.001</td>
<td>0.18</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>167</td>
<td>170</td>
<td>173</td>
<td>7</td>
<td>0.30</td>
<td>0.02</td>
<td>0.22</td>
</tr>
<tr>
<td>Leucine</td>
<td>327</td>
<td>333</td>
<td>343</td>
<td>17</td>
<td>0.07</td>
<td>0.03</td>
<td>0.15</td>
</tr>
<tr>
<td>Lysine</td>
<td>87.7</td>
<td>90.0</td>
<td>88.6</td>
<td>2.7</td>
<td>0.67</td>
<td>0.009</td>
<td>0.26</td>
</tr>
<tr>
<td>Methionine</td>
<td>32.7(^A)</td>
<td>33.0(^A)</td>
<td>30.1(^B)</td>
<td>0.8</td>
<td>0.001</td>
<td>0.001</td>
<td>0.16</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>61.6</td>
<td>61.6</td>
<td>63.3</td>
<td>1.5</td>
<td>0.83</td>
<td>0.42</td>
<td>0.08</td>
</tr>
<tr>
<td>Proline</td>
<td>110</td>
<td>115</td>
<td>113</td>
<td>4</td>
<td>0.37</td>
<td>0.001</td>
<td>0.39</td>
</tr>
<tr>
<td>Serine</td>
<td>91.5</td>
<td>96.1</td>
<td>94.0</td>
<td>2.5</td>
<td>0.11</td>
<td>0.001</td>
<td>0.38</td>
</tr>
<tr>
<td>Threonine</td>
<td>104</td>
<td>107</td>
<td>103</td>
<td>4</td>
<td>0.27</td>
<td>0.07</td>
<td>0.48</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>38.5</td>
<td>38.5</td>
<td>39.6</td>
<td>1.0</td>
<td>0.33</td>
<td>0.001</td>
<td>0.52</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>67.5</td>
<td>67.8</td>
<td>70.7</td>
<td>3.1</td>
<td>0.17</td>
<td>0.10</td>
<td>0.16</td>
</tr>
<tr>
<td>Valine</td>
<td>403</td>
<td>416</td>
<td>421</td>
<td>13</td>
<td>0.053</td>
<td>0.12</td>
<td>0.24</td>
</tr>
</tbody>
</table>
## Table 4. Ingredient composition and analyzed nutrient content of the experimental ration

**Experiment 2**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>47.92</td>
</tr>
<tr>
<td>Triticale silage</td>
<td>8.96</td>
</tr>
<tr>
<td>Ground corn grain</td>
<td>15.65</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>7.35</td>
</tr>
<tr>
<td>Canola meal</td>
<td>7.29</td>
</tr>
<tr>
<td>Dehydrated citrus pulp</td>
<td>7.17</td>
</tr>
<tr>
<td>Rumen bypass fat&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.38</td>
</tr>
<tr>
<td>Sugar byproduct&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.02</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.82</td>
</tr>
<tr>
<td>Mineral and vitamin mix&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.46</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.50</td>
</tr>
<tr>
<td>Urea</td>
<td>0.45</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.39</td>
</tr>
<tr>
<td>Rumen protected lysine&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.27</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>0.21</td>
</tr>
<tr>
<td>Potassium magnesium sulfate</td>
<td>0.18</td>
</tr>
<tr>
<td>DM, %, ± SD</td>
<td>48.3</td>
</tr>
</tbody>
</table>

**Nutrient, % DM ± SD**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>% DM ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>15.4</td>
</tr>
<tr>
<td>NDF</td>
<td>29.2</td>
</tr>
<tr>
<td>ADF</td>
<td>18.3</td>
</tr>
<tr>
<td>Starch</td>
<td>27.5</td>
</tr>
<tr>
<td>Ash</td>
<td>7.0</td>
</tr>
<tr>
<td>NE&lt;sub&gt;L&lt;/sub&gt;</td>
<td>0.79</td>
</tr>
</tbody>
</table>

<sup>1</sup>MEGALAC (Church & Dwight Co., Inc, Princeton, NJ).

<sup>2</sup>Contained 92.3% sucrose (Renaissance Nutrition Inc., Roaring Spring, PA).

<sup>3</sup>Contained 14.8% calcium, 34.0% magnesium, 0.8% sulfur, 105 mg/kg Fe, 4,213 mg/kg Zn, 817 mg/kg Cu, 4,200 mg/kg Mn, 65.1 mg/kg Se, 141 mg/kg Co, 191 mg/kg I, 882 KIU/kg Vitamin A, 220 KIU/kg Vitamin D, and 5,292 IU/kg Vitamin E.

<sup>4</sup>LysiGEM
Table 5. Intake and production responses for all cows (multiparous, n=22, and primiparous, n=4) that were retained in the model which were provided either a control diet deficient in MP methionine by approximately 17 g, or control diet supplemented with either 13 g of MP Met from KESSENT M\(^1\) (RPM-K), or Smartamine M\(^2\) (RPM-S).

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th></th>
<th></th>
<th>SEM</th>
<th>RPM(^3)</th>
<th>Source(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON RPM-S M</td>
<td>RPM-K</td>
<td></td>
<td></td>
<td>RPM(^3)</td>
<td>Source(^3)</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>26.3</td>
<td>26.6</td>
<td>26.2</td>
<td>0.79</td>
<td>0.61</td>
<td>0.02</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>38.3</td>
<td>38.4</td>
<td>38.1</td>
<td>1.4</td>
<td>0.85</td>
<td>0.35</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.60</td>
<td>3.66</td>
<td>3.69</td>
<td>0.17</td>
<td>0.02</td>
<td>0.47</td>
</tr>
<tr>
<td>Fat, kg/d</td>
<td>1.37</td>
<td>1.40</td>
<td>1.39</td>
<td>0.06</td>
<td>0.07</td>
<td>0.74</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.25</td>
<td>3.28</td>
<td>3.28</td>
<td>0.1</td>
<td>0.04</td>
<td>0.98</td>
</tr>
<tr>
<td>Protein, kg/d</td>
<td>1.24</td>
<td>1.25</td>
<td>1.24</td>
<td>0.05</td>
<td>0.26</td>
<td>0.39</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.91</td>
<td>4.89</td>
<td>4.88</td>
<td>0.03</td>
<td>0.04</td>
<td>0.78</td>
</tr>
<tr>
<td>MUN, mg/dL</td>
<td>8.28</td>
<td>8.36</td>
<td>8.24</td>
<td>0.28</td>
<td>0.86</td>
<td>0.43</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>39.7</td>
<td>40.3</td>
<td>40.0</td>
<td>1.23</td>
<td>0.18</td>
<td>0.51</td>
</tr>
<tr>
<td>ECM/DMI, kg/kg</td>
<td>1.51</td>
<td>1.52</td>
<td>1.53</td>
<td>0.05</td>
<td>0.25</td>
<td>0.37</td>
</tr>
<tr>
<td>Somatic cell score</td>
<td>1.91</td>
<td>1.92</td>
<td>2.03</td>
<td>0.40</td>
<td>0.74</td>
<td>0.59</td>
</tr>
<tr>
<td>Bodyweight, kg</td>
<td>714.5</td>
<td>718.3</td>
<td>716.6</td>
<td>16.1</td>
<td>0.31</td>
<td>0.54</td>
</tr>
</tbody>
</table>

\(^1\)KESSENT M (Kemin Industries Inc., Des Moines, IA).
\(^2\)Smartamine M (Adisseo USA Inc., Alpharetta, GA).
\(^3\)Contrasts evaluated the overall effect of providing rumen protected methionine (RPM; CON vs. RPM-S vs. RPM-K) and the comparison of RPM-S to RPM-K (Source; RPM-S vs. RPM-K).
Table 6. Plasma amino acid concentrations for all cows (multiparous, n=22, and primiparous, n=4) that were retained in the model which were provided either a control diet deficient in MP methionine by approximately 17 g, or control diet supplemented with either 13 g of MP Met from KESSENT M\(^1\) (RPM-K), or Smartamine M\(^2\) (RPM-S).

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>CON</th>
<th>RPM-S</th>
<th>RPM-K</th>
<th>SEM</th>
<th>P Values</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met</td>
<td>31.6</td>
<td>45.2</td>
<td>45.2</td>
<td>2.24</td>
<td>&lt;0.0001</td>
<td>0.97</td>
</tr>
<tr>
<td>Ala</td>
<td>294.0</td>
<td>301.0</td>
<td>302.0</td>
<td>12</td>
<td>0.21</td>
<td>0.89</td>
</tr>
<tr>
<td>Arg</td>
<td>79.2</td>
<td>77.3</td>
<td>79.0</td>
<td>3.78</td>
<td>0.65</td>
<td>0.52</td>
</tr>
<tr>
<td>Asn</td>
<td>56.8</td>
<td>55.7</td>
<td>57.0</td>
<td>2.23</td>
<td>0.79</td>
<td>0.52</td>
</tr>
<tr>
<td>Asp</td>
<td>5.3</td>
<td>5.1</td>
<td>5.2</td>
<td>0.19</td>
<td>0.25</td>
<td>0.57</td>
</tr>
<tr>
<td>Glu</td>
<td>46.2</td>
<td>45.6</td>
<td>45.6</td>
<td>1.78</td>
<td>0.41</td>
<td>0.95</td>
</tr>
<tr>
<td>Gln</td>
<td>269.2</td>
<td>267.9</td>
<td>275.7</td>
<td>11.05</td>
<td>0.66</td>
<td>0.25</td>
</tr>
<tr>
<td>Gly</td>
<td>305.7</td>
<td>289.0</td>
<td>291.8</td>
<td>12.5</td>
<td>0.06</td>
<td>0.77</td>
</tr>
<tr>
<td>His</td>
<td>60.4</td>
<td>61.0</td>
<td>60.6</td>
<td>2.83</td>
<td>0.77</td>
<td>0.81</td>
</tr>
<tr>
<td>Ile</td>
<td>129.9</td>
<td>125.7</td>
<td>125.7</td>
<td>6.32</td>
<td>0.18</td>
<td>0.99</td>
</tr>
<tr>
<td>Leu</td>
<td>162.0</td>
<td>157.1</td>
<td>158.2</td>
<td>8.44</td>
<td>0.26</td>
<td>0.80</td>
</tr>
<tr>
<td>Lys</td>
<td>96.9</td>
<td>96.6</td>
<td>98.0</td>
<td>5.76</td>
<td>0.91</td>
<td>0.71</td>
</tr>
<tr>
<td>Phe</td>
<td>44.2</td>
<td>42.6</td>
<td>43.2</td>
<td>1.67</td>
<td>0.17</td>
<td>0.61</td>
</tr>
<tr>
<td>Pro</td>
<td>88.8</td>
<td>88.6</td>
<td>90.2</td>
<td>2.87</td>
<td>0.74</td>
<td>0.50</td>
</tr>
<tr>
<td>Ser</td>
<td>81.6</td>
<td>77.7</td>
<td>79.9</td>
<td>2.31</td>
<td>0.24</td>
<td>0.41</td>
</tr>
<tr>
<td>Thr</td>
<td>147.7</td>
<td>143.9</td>
<td>145.5</td>
<td>8.28</td>
<td>0.38</td>
<td>0.68</td>
</tr>
<tr>
<td>Trp</td>
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<td>37.2</td>
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\(^1\)KESSENT M (Kemin Industries Inc., Des Moines, IA).

\(^2\)Smartamine M (Adisseo USA Inc., Alpharetta, GA).

\(^3\)Contrasts evaluated the overall effect of providing rumen protected methionine (RPM; CON vs. RPM-S vs. RPM-K) and the comparison of RPM-S to RPM-K (Source; RPM-S vs. RPM-K).
REFERENCES


69


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Osorio JS, Ji P, Drackley JK, Luchini D, Loor JJ. Smartamine M and MetaSmart supplementation during the peripartal period alter hepatic expression of gene


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estimate the methionine bioavailability of two forms of 2-hydroxy-4-methylthiobutanoic acid (HMB) for lactating cows. J. Dairy Sci. 84(Suppl.1):35. (Abstr.).


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Appendix

IACUC PROTOCOL APPROVAL

University of Delaware
Institutional Animal Care and Use Committee
Application to Use Animals in Research
(New and 3-Yr submission)

Title of Protocol: Evaluating the availability of rumen-protected amino acid products

AUP Number: 52R-2018-0

Principal Investigator: Tanya Gressley

Common Name (Strain/Breed if Appropriate): Holstein cows

Genus Species: Bos taurus

Date of Submission: 5/29/18

Official Use Only

IACUC Approval Signature: [Signature]

Date of Approval: 6/20/18
**Title of Protocol:** Animal performance response to two rumen protected methionine products

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**Date of Submission:** Click here to enter text.

**Official Use Only**

IACUC Approval Signature: [Signature]

Date of Approval: 11/5/19