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Lactation performance and nitrogen efficiency of dairy cows fed increasing amounts of microencapsulated methionine

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Lactation performance and nitrogen efficiency of dairy cows fed increasing amounts of microencapsulated methionine

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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DEDICATION

This thesis is written in dedication to my parents, Danny and Dawn King. Dad, thank you for showing me the value in hard work and how to be dedicated to my studies. Mom, your endless support and friendship has been a blessing throughout my graduate program. I love you both! Proverbs 3:6.

Layla Eve

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ABSTRACT

Amino acid requirements of high-producing dairy cows may be greater than what dietary and microbial crude protein can provide. Therefore, study objectives were to determine lactation performance of dairy cows fed rumen-protected methionine (**MET**; Timet®, VETAGRO, Italy). Individually-fed, multiparous Holstein cows (n = 48, 127 ± 41 DIM and 671 ± 8 kg BW) were used in a replicated 4 × 4 Latin square design with 28-d periods. Dietary treatments consisted of a basal TMR deficient in metabolizable MET (-10 g/cow/day): 1) Control (**CON**) with no supplement; 2) low methionine (**LM**) diet with 11 g/cow/d MET; 3) medium methionine (**MM**) containing 19.25 g/cow/d MET; and 4) high methionine (**HM**) diet plus 27.5 g/cow/d MET. Milk yield and feed intake data from the last 7 d of each period were used for analyses; BW, BCS and milk components were determined on d 27 and 28 of each period. Statistical analyses were performed using the MIXED procedures of SAS with square, period within square, and treatment as fixed effects and cow within square as a random effect. Linear, quadratic and cubic effects were also tested. No evidence of quadric or cubic effects was observed for any of the response variables. Dry matter intake was similar ($P = 0.64$) across treatments whereas milk production increased linearly from 38.0 ± 0.87 kg/d for CON to 39.7 ± 0.87 kg/d for HM. Milk fat concentration was similar across treatments ($P = 0.83$) averaging 3.69 ± 0.88% while milk protein concentration tended to increase linearly ($P = 0.11$) from 3.17 ± 0.04% for CON to 3.21 ± 0.04% with high MET supplementation. Overall, supplementation with MET resulted in greater ($P \leq 0.01$) yield of milk protein and fat. Consequently, yield of energy-corrected milk (**ECM**) increased linearly; cows consuming the CON diet produced 38.3 ± 1.05 kg ECM/d whereas MET supplementation resulted in 41.4, 40.9 and 41.7 ± 1.05 kg ECM/d, for LM, MM and HM, respectively. Concentration of MUN averaged 13.5 ± 0.23 mg/dL across treatments ($P = 0.58$). These data suggest that the MET supplement increased the supply of metabolizable methionine resulting in increased yield of milk and milk components.

CHAPTER 1: LITERATURE REVIEW

Introduction

Dietary protein normally refers to crude protein (CP) of the diet which is chemically defined as the nitrogen (N) content. Nitrogen typically accounts for 16% of the protein content (NRC, 2001), so every gram of measured nitrogen represents approximately 6.25 g of crude protein.

Dairy cattle and other ruminant animals utilize dietary and rumen microbial crude protein as sources of amino acids (AA) (Schwab and Broderick, 2017). Dietary protein is broken down by proteolytic enzymes from bacteria and protozoa in the rumen to individual amino acids (AA), N, volatile fatty acids (VFA), and CO_2. The nitrogen is combined with other VFA for the synthesis of new amino acids, which are then used for synthesis of microbial crude protein (MCP). Dietary crude protein is classified as either rumen undegradable protein (RUP) or rumen degradable protein (RDP) made of almost all non-protein nitrogen (NPN) and rest of the true protein. The RUP will bypass rumen degradation and be digested in the small intestine, while RDP is digested by microbes in the rumen and incorporated into microbial protein, which is also passed to the small intestine and digested in the same manner as RUP. Factors that affect ruminal degradation of dietary proteins include physical barriers (cell walls, cross linking), feed intake, and types of feed processing. For example, heat can enhance formation of complexes between protein and carbohydrates and thus reduce digestibility.

The rumen is able to provide microbial protein which is built off of RDP that are broken down to single AA to build peptides for the animal's use. Free AA are not absorbed in the rumen but will group together (up to 5 AA) and be taken into bacterial cells for facilitated transport by a carrier. AA can also be metabolized for energy if there are not enough carbohydrates to maintain animal growth. Higher producing dairy cattle have an AA requirement that is often greater than what RUP and MCP are able to provide, especially for lysine (Lys) and methionine (Met). Research suggests that Met and Lys are consistently the first limiting AA in lactating dairy cow diets (NRC, 2001; Doepel et al., 2004; Lapierre et al., 2006). Even though feedstuffs contain the nutrients to make these AA readily available to the animal, rumen degradability is a major concern. Feedstuffs can be treated (heat or chemical) to reduce protein's degradability in the rumen. In addition, supplementation with essential AA is often used as a strategy to meet these increased AA requirements, although microbial degradation of free AA in the rumen impairs the response to this strategy.

Alternatively, producers often overfeed RUP in an attempt to meet the essential AA requirements. This strategy is wasteful, expensive and leads to inefficient nitrogen utilization. The excess nitrogen resulting from overfeeding protein is ultimately metabolized to urea, which is an energetically costly process and can lead to reduced cow body condition scores (Reed et al., 2017). The increased weight loss can be offset by increasing fat content in the diet; however, this results in an increased cost to producers. In addition, the excess nitrogen can also have detrimental impacts on the environment. The nitrogen excreted in dairy manure can lead to nitrous oxide and ammonia production in livestock housing, in manure storage facilities, and following application to the land. Nitrous oxide adds to eutrophication through run-offs and can adversely impact crop growth if over-applied to soils (Johnson et al., 2016).

In addition to environmental concerns with nitrogen, dietary protein is an expensive dietary nutrient, representing around 42% of the cost in a lactating cow diet. To avoid the consequences of overfeeding protein, and since dietary protein and MCP are insufficient for higher producing dairy cattle, rumen-protection strategies can be employed to provide a more stable supply of these essential AA.

Heat or chemical treatment, microencapsulation, AA analogs, and esophageal groove closure are all examples of rumen-protection methods for feedstuffs. Microencapsulation proves to be the most reliable and stable method to bypass the rumen. Microencapsulation is the process of enclosing micron-sized particles in a polymeric shell that protects the core material from microbial degradation (Jyothi et al., 2010). Liposome microencapsulation, spray drying, spray cooling, centrifugal coextrusion, rapid expansion of supercritical solutions and extrusion are different microencapsulation techniques that are utilized depending on the desired outcome. These rumen protection methods offer a functional method to supply the necessary metabolizable Met needed by high-producing cows. Additionally, diet formulations with rumen protected AA allow those diets to have lower total crude protein, potentially increasing the efficiency of nitrogen use and reducing nitrogen excretion into the environment (Rogers et al., 1987; Leonardi et al., 2003).

Protein Metabolism

The National Research Council (NRC, 2001) recommends that diets contain 17.5% to 19.0% dietary crude protein (CP) to support high milk production. Dairy cattle tissues undergo dramatic changes in metabolism in the first weeks of lactation. For example, protein mass in the tissues of the mammary gland, rumen, small and large intestine are significantly

increased when compared to dry cows (Bequette et al., 1998). After calving, milk protein secretion significantly increases relative to feed intake. Consequently, the cow would be forced to utilize body protein reserves, primarily the muscle proteins for fulfilling both amino acid (the precursors) and energy requirements of high rates of milk protein output. The amino acids mobilized from muscle tissue proteins can also contribute to gluconeogenesis in the liver. Thus, it is essential to provide lactating animals sufficient amounts of supplemental dietary AA to counteract this increase in body protein utilization.

In ruminants, N losses are affected by many factors such as crude protein content of the diet, DMI, diet type, and production status of animal. In cattle consuming diets formulated for amino acids and ammonia, N levels can surpass apparent digestibility of N, resulting in a negative N balance. This negative balance can be corrected by the return of urea to the rumen to aid in microbial protein synthesis and thus supply of AA to the animal. Between 40 and 80% of urea-N synthesized by the liver is delivered back to the rumen where 35 to 55% of it is converted to further use in ruminant animals (Lapierre and Lobley, 2001). Even though ruminant animals overall are inefficient N utilizers, they are highly efficient in terms of urea recycling which is used as a N source for rumen microbes. Supplementation of RDP can be used to support urea recycling for cattle eating low quality forages. In fact, feeding RDP with low quality forages can improve forage utilization (Köster, 1996) and animal performance (Mathis et al., 1999). The increased metabolic activity of rumen microbes will improve the energy status of the animal and ultimately increase N flow to the duodenum (Wickersham et al., 2008). Wickersham et al. (2008) fed a low-quality prairie-hay (4.9% CP) *ad libitum* to steers with increasing amounts of RDP postulating that urea recycling would make up the difference in quality of forage. As expected, with increasing

amounts of RDP, NDF digestibility was improved, urinary N excretion increased, N intake increased, and fecal N increased. However, as RDP increased in the diet, a decrease in urea production entering the gut was also observed. The more RDP that was provided to the animals, the less urea was recycled back to the gut. With low quality forage and low amounts of RDP supplied these researchers reported a 98.9% rate of conservation showing the remarkable ability of cattle to conserve N when experiencing a stark N deficiency (Wickersham et al., 2008).

Furthermore, in situations where dietary protein and MCP are not enough to meet the animal's AA requirements, urea recycling and feeding higher levels of CP in diet are production methods to attempt meeting those requirements. However, high CP levels in the diet are costly to a producer, can become metabolically wasteful, and will increase N excretion. The most direct way to reduce N excretion and meet AA requirements is to reduce dietary N but when that is done production decreases. In order to avoid these consequences, bypass AA can be supplemented depending on the limiting AA in the diet.

Limiting Amino Acids

Histidine

Histidine (His) is an essential alpha-amino acid that is limiting in milk production when dairy cows are fed grass silage-based diets with low amounts of plant proteins. Normally, Met and Lys tend to be the first limiting AA in corn silage and alfalfa haylage-based diets, but His may also limit milk production in these different feeding scenarios (Kim et al., 1999; Vanhatalo et al., 1999). Lee et al. (2012) fed metabolizable protein deficient diets and observed a significant decrease in plasma concentrations of His which was correlated

with a lower microbial protein concentration of His. It was theorized that His is a limiting AA for milk production and positive effects of supplementation of rumen-protected His to a diet balanced for Met and Lys were observed. In this study, an increase in milk yield and protein concentration were observed with the supplementation of RPHis. In support of the findings of Kim et al. (1999), Korhonen et al. (2000) and Huhtanen et al. (2002) also assumed that His is the first limiting AA when the diet is grass silage based or low RUP amounts. Additional studies have been conducted (Doelman et al., 2008; Aines et al., 2010) to test the effects of supplemental His in corn silage and alfalfa-based diets; these yielded inconclusive data. It is important to note that the diets in these studies were high in protein and the likelihood of His truly being limiting was unlikely. These studies were also conducted over relatively short periods of time, which may not have given a sufficient amount of time for a His deficiency to become apparent.

Arginine

Arginine (Arg) is widely described as a conditionally essential AA which is absorbed by the mammary gland and used for cellular and milk protein synthesis (Tian et al., 2017). Research on the supplementation of Arg has mainly focused on monogastric animals. For example, Mateo et al. (2002) supplemented Arg and reported that it increased litter weight gain of primiparous sows as well as sow milk production. In addition, 2 to 3 times more Arg is absorbed by the mammary gland than what will appear in milk protein (Mepham, 1982). The functions of Arg include regulation of endocrine secretion and mammary secretions, improved immunity and digestive function, promotion of self-repairing capabilities, and

improved anti-oxidative capacity (Wu et al., 2009). In ruminant animals, supplementation with Arg increases casein synthesis in bovine mammary epithelial cells (Wang et al., 2014).

Ding et al. (2019) conducted a study with six lactating Holstein cows supplemented with Arg through jugular infusions to investigate the effects on milk yield, milk protein, and nitrogen utilization efficiency. Infusion of Arg led to no significant differences in milk protein concentration, milk yield or milk efficiency. However, the infusion of Arg did increase the milk protein yield when compared to control animals. Furthermore, no improvement in nitrogen utilization efficiency was observed.

A more significant response is observed situations where there is a deficiency of an AA from a balanced profile than from the supplementation of an AA (Lapierre et al., 2009). Tian et al. (2014) removed Arg from the essential AA profile in a diet that had adequate methionine and lysine to test the effects on milk yield and milk protein yield. Treatments included jugular infusions of a control saline, complete essential AA profile, complete profile without leucine, and a complete profile without Arg. The cows receiving the complete essential AA profile had the highest milk yield and milk components. When Arg was removed from the AA profile, milk yield and components decreased, confirming the hypothesis that Arg is crucial for the support of milk production and milk components.

Arginine is the precursor for nitric oxide and polyamine compounds in the body (Wu and Morris, 1998). Nitric oxide has positive effects on blood flow regulation enhancing nutrient uptake, fetal development, lactation, and ovarian function (Lefèvre et al., 2011). In both monogastric and ruminant animals, Arg supplementation increases embryonic and fetal survival (Mateo et al., 2007; Lassala et al., 2011). Meyer et al. (2018) observed that rumen-

protected Arg increased the delivery of Arg to small intestine when compared to intravenous infusions.

Lysine

Lysine (Lys) and methionine are often recognized as the first limiting AA for milk production in dairy cattle, particularly when corn-based diets are fed (Polan et al., 1991). There are fewer rumen-protected lysine products when compared to rumen-protected methionine products possibly due to the fact that the lysine formulations are more expensive and less stable (Swanepoel et al., 2010). Typically, only small amounts of Lys actually escape rumen degradation (Velle et al., 1998; Robinson et al., 2006) suggesting that some form of rumen-protected Lys is necessary to supply adequate amounts of Lys to the cow's small intestine. The NRC recommends diets contain 7.2% Lys of the metabolizable protein to maximize milk protein percentage and yield. For example, cattle diets that include corn distillers dried grains with solubles (DDGS) tend to be deficient in lysine because DDGS have low levels of Lys and relatively high CP (30% DM) levels (Schingoethe et al., 2009; Kelzer et al., 2010). The low Lys levels in diets including DDGS suggest more RUP should be fed to meet NRC recommendation which will lead to excess N being excreted (Paz et al., 2013). Paz et al. (2013) evaluated the effects of rumen-protected Lys supplementation fed to lactating dairy cows consuming diets including DDGS and concluded that supplementation with rumen-protected Lys had no beneficial effect. Supplementation with Lys decreased plasma concentrations of Lys, Arg, His, and Val was observed. Despite the low Lys levels when feeding DDGS there was still sufficient amounts to support milk percentage and yield. In addition, Swanepoel et al. demonstrated that feeding rumen-protected lysine to lactating dairy cows resulted in a decrease in blood plasma levels of most AA, suggesting that the diet

used in Swanepoel's study had lysine as the first limiting AA. No positive response in milk protein was observed and there was a significant decrease in plasma 3-methylhistidine concentrations, implying muscle protein synthesis may have been stimulated. Swanepoel et al. (2016) concluded that supplementation of rumen-protected Lys was not beneficial and could ultimately lead to negative outcomes.

Alternatively, positive effects have been noted in other studies (Polan et al., 1991; Bailey et al., 2019). Polan et al. (1991) observed that supplementation with Lys led to an increase in milk yield and milk protein yields in dairy cows fed corn gluten meal-based diets. However, when Lys was supplemented to cows fed a soybean meal-based diet, there were insufficient differences documented to recommend supplemental Lys. Bailey et al. (2019) supplemented Lys to a basal diet deficient in metabolizable Lys. They concluded that supplemental Lys supported an increase in milk production and could be used to benefit dairy cattle fed corn-based diets. In conclusion, supplemental Lys in diets fed to dairy cattle tends to only be beneficial when those diets are deficient in metabolizable Lys.

Methionine

Responses of milk protein to changes in metabolizable protein supply are often marginal because the conversion of dietary N into milk has a low efficiency (25-30%). Although efficiency can be improved by decreasing crude protein levels in diet, this adjustment can sacrifice milk yields and protein concentrations, which can be reversed by supplementing dietary proteins with rumen protected or postruminal infusions of essential amino acids (Zhao et al., 2019). However, responses observed from those supplementation techniques are often unpredictable and are lower than what is usually predicted (Bequette et al., 1998). Responses to AA supply are dependent upon stage of lactation which is associated

with significant changes in muscle protein turn-over rates, which may alter the availability of AA to the mammary gland. For example, efficiency of converting additional essential AA into the milk is higher in early lactation than in mid-lactation. In addition, milk protein concentration is increased more when AA are infused intravenously when compared to being ingested (Bequette et al., 1998).

Methionine is a limiting essential AA for lactating dairy cows fed legume forages, corn silage, corn grain and soybean meal (Schwab et al., 1976; NRC, 2001). Broderick et al. (2008) conducted a trial supplementing rumen-protected methionine (RPM; Mepron) and suggested that supplementation allows feeding less crude protein. Supplemental Met increases milk yield (Schmidt et al., 1999), milk protein concentration (Armentano et al., 1997; Zhao et al., 2019) and milk protein yield (Rulquin and Delaby, 1997). However, Leonardi et al. (2003) reported conflicting data with that supplementation of RPM with two diet levels of crude protein (16.1% to 18.8%). No interactions between crude protein level and Met supplementation were observed. Similarly, Met did not affect milk production, milk protein yield, or N excretion in urine or feces.

Berthiaume et al. (2001) fed five lactating dairy cows a basal TMR that was top dressed with 0 or 72 g of rumen-protected methionine per day. The bypass ability of the methionine was 66% and 82% of that amount was absorbed in the small intestine. Arterial concentrations of Met significantly increased as dietary Met increased along with milk lactose concentration. Milk yield and concentration of fat, protein and casein were unaffected. Furthermore, higher concentrations of urea-N and glucose in arterial plasma were observed with no effect on insulin contrasts. Met interacts with branch chained AA by using the same dehydrogenase complex in the transamination pathway, resulting in oxidation of

AA. Thus, it was assumed Ala and Asp were oxidized and produced glucose and resulted in synthesis of urea. The increase in glucose is responsible for the increased amount of lactose in the milk. Following the trend reported in other studies, milk production and milk composition were unaffected.

As previously mentioned, lysine and methionine are usually the first limiting AA in dairy cattle diets. Armentano et al. (1997) conducted an experiment where milk production response of Holsteins cows fed rumen-protected methionine at two levels were determined. Sixteen Holstein cows in early lactation were used in a replicated 4 × 4 Latin square design with 21-d periods. The basal diet was based on alfalfa and heated soybeans. Within this study they found that with each additional gram of supplemental methionine, milk protein yield increased by 4 g and protein concentration increased as well. In other trials with corn silage as the primary forage, increases in milk protein yield and concentration were observed in response to RPM. Armentano et al. (1997) reported a higher mean milk production in response to lysine addition to the basal TMR. In addition, Zhao et al. (2019) fed ten lactating Holstein cows a high protein diet (16%), a low protein diet (12%), and supplemented AA (Met, Thr, Ile, and Leu) to the low protein diet. The supplementation of RPM to the low protein diet increased milk yield, milk protein concentration, milk urea N, and nitrogen efficiency. However, there were no significant improvements in dietary N digestibility, which suggests that the improvement in N efficiency was caused by an adjustment to the absorption of AA toward milk protein synthesis. These studies support the earlier statement that a diet containing a complete essential AA profile is more beneficial to the host animal than specifically selecting one AA to supplement.

Nitrogen Efficiency

An increase in CP will result in a decrease in N efficiency and an increase in N excretion. Ruminant animals are relatively inefficient in terms of N utilization and will excrete 65 to 80% of dietary N intake via urine and feces resulting in environmental pollution. Furthermore, Liu and VandeHaar (2020) made a connection between residual feed intakes and protein efficiency. It was observed that dairy cows with lower feed intakes utilize protein more efficiently than those who consume more. However, higher producing animals with greater feed intakes will need further support to improve nitrogen efficiency.

The manure from dairy farms has been recognized as a major source of ammonia emissions (Külling et al., 2001). A major portion of nitrogen in manure is excreted in the form of urea and can be converted to ammonia through a bacterial degradation by the enzyme urease. The release of ammonia from the manure is dependent on the concentration of nitrogen. This relationship is linear – For example, an increase in the nitrogen concentration in manure will increase ammonia emissions (Elzing and Monteny, 1997). All food production systems can lead to negative agroecosystem effects such as ozone, groundwater, stream, river and soil (Cowling and Galloway, 2002). For example, the ammonia contributes to water eutrophication, aerosol formation, soil acidification, and impaired visibility (USEPA, 2004a). Damage is caused through acid deposition to the ecosystem and also represents a loss of the value of manure as a fertilizer. Ammonia emissions are regulated by the United States Environmental Protection Agency (USEPA) in response to the Clean Air Act (USEPA, 1990). Since farm animals are the greatest contributor to ammonia emissions in the United States (NRC, 2003), dairy farms with 700 or

more cows are encouraged to sign an EPA Air Quality Compliance Agreement or must notify emergency officials if more than 45 kg of ammonia or hydrogen sulfide is emitted (Hristov, 2011). This agreement excludes reports of normal manure handling but is required to state other forms of release such as a spill or burst from an ammonia tank. Ammonia emissions can lead to the formation of fine particulate matter (particles with aerodynamic diameter ≤ 2.5 μm, USEPA, 2004b) which is considered dangerous when inhaled because it may reach bronchioles and interfere with gas exchange (Hristov, 2011). Significant amounts of ammonia emissions come from urinary N in the form of urea. Depending on management techniques, 30 to 50% of N in urine and feces is released as ammonia (Hristov et al., 2009).

The nitrogen concentration of manure can be manipulated in the cattle's diet, which has been widely proven (Chalupa and Ferguson, 1996; Chase, 1999; Godden et al., 2001). It has been postulated that supplementing a bypass AA in a dairy cow's diet can aid in improving N excretion and efficiency. Broderick et al. (2008) supplemented Met (RPM, Mepron) to lower crude protein in diet, which would result in reduced urinary N excretion. As expected, milk urea nitrogen (MUN) was reduced and was paralleled by N efficiency being increased. The apparent N efficiency was improved by 8% from highest CP level to lowest. The diet containing the lowest level of CP had the greatest improvement in efficiency. Thus, it was possible to feed a diet with CP levels as low as 16.1% without a reduction in milk production or milk components.

In conclusion, providing supplemental bypass AA to deficient diets can significantly improve milk production, protein concentration, and has the ability to improve nitrogen utilization efficiency. Various rumen-protection methods such as heat or chemical treatment,

microencapsulation, AA analogs, and esophageal groove closure are available to supply these bypass AA's. Microencapsulation is the most commonly used technique with fewer limitations.

Microencapsulation Techniques

Spray Drying

The process of spray drying has been in used since the 1950s. The initial goal of spray drying microencapsulation was to give flavorful oils protection against degradation or oxidation. Spray drying now has the ability to encapsulate sugars, polysaccharides, starches, proteins, vitamins, pigments and leavening agents. During the drying process, evaporation of the solvent and entrapment of core material is rapid which limits the shell materials to those that can withstand this process. Gum acacia, maltodextrins, and hydrophobically modified starch are typically used as shell materials. Furthermore, proteins and other polysaccharides can also be used for shell material, but these materials are more expensive and require more labor because of their lower solubility in water. No new shell materials have been introduced over the last 20 years (Beristain et al., 1999).

Normally, spray-drying is considered a dehydration process, but it also has the ability to encapsulate material to form a polymer or melt. The spray drying process has four stages: preparation of the dispersion or emulsion; homogenization of the dispersion; atomization of the infeed emulsion; and dehydration of the atomized particles. Feed temperature, air inlet temperature and outlet temperature are the primary factors that must considered to conduct this process successfully. Explosions and related risks need to be mitigated due to the number

of volatiles and high temperatures used during this process. Overall, this is the most commonly used technique to encapsulate food materials because of its cost effectiveness. However, it is not be energy efficient because of the underutilization of the heat passing through the drying chamber (Gouin, 2004; Gharsallaoui et al., 2007).

Spray Cooling/Chilling

Spray cooling combined with chilling is considered to be the least expensive encapsulation technology. This process can be used to encapsulate organic salts, inorganic salts, textural ingredients, enzymes, and flavors. It improves heat stability, delays a timed or controlled release in damp environments, and can turn a liquid ingredient into a powder. In order to achieve this process a matrix microcapsule is formed, which is not considered a "true" microencapsulation. The core material is "buried" in a fat matrix which allows active ingredients to be on the surface having direct access to its surroundings. Given a significant amount of active ingredient is on the surface of the microcapsule, the controlled release may occur within a few minutes after interacting with food stuff. However, even though the process does not produce a true capsule, the properties are sufficient to create a delayed release. A way to prevent rapid release would be to strengthen the binding of the ingredient to the fat matrix even though the matrix may be damaged during the process. To achieve a stronger bond, fine tuning of the kinetic release can be improved with changing the crystalline structure of various shell materials (Gouin, 2004).

Centrifugal Coextrusion

The centrifugal coextrusion microencapsulation process is classified as an atomization method that is used to modify spray cooling/chilling. It utilizes a modified double fluid nozzle to pump out an active ingredient. The active ingredient or the core material will be pumped through the inner part of the nozzle while the shell/wall material is run through the outer part of the nozzle. Rayleigh instabilities at the edge of the nozzle aids in forming a bead twice the size of the nozzle's diameter. Rayleigh instabilities create an interface which allows two materials with different densities to bind together. An advantage to this process is that the release of the coextruded product is slower when compared to spray cooling. This process gives more leeway for a more delayed response. However, on a large-scale production, this limitation of this process is that it is engineering intensive (Gouin, 2004).

Rapid Expansion of Supercritical Solutions (RESS)

The RESS microencapsulation process uses supercritical fluids for the encapsulation. A supercritical fluid is a dense solvating gas or a low-viscosity, low density liquid. Substances that can be manipulated to be in a supercritical state are carbon dioxide, water, propane, nitrogen, and others. The most common supercritical substance utilized in the microencapsulation process is carbon dioxide because it is the second most abundant and least expensive substance. These supercritical fluids are able to encapsulate heat-sensitive material which uses the same equipment (nozzle and spray tower) as spray drying. The main benefit of the use of supercritical fluid is the lack of water and the process uses lower temperatures. This gives microencapsulation the opportunity to encapsulate very volatile

flavors, sensitive ingredients, and enzymes. The RESS process occurs when a supercritical solvent containing shell material is pressurized resulting in the active ingredient is being released through a small orifice, leading to a pressure drop causing the desolvation of a shell material to form around the active ingredient. This technique allows for manipulation of the outer shell layer thickness between 100/um and a monomolecular layer (Gouin, 2004). Within this process there are different methods; these methods vary based on how quickly the supercritical fluid is cooled or they use lower temperatures which leads to better control over the microcapsules (Ribeiro et al., 2003; Thies et al., 2003).

Extrusion

The extrusion microencapsulation process has previously only been utilized for encapsulating volatile and unstable flavors inside a glassy carbohydrate matrix. The glassy hydrophilic matrix provides a long shelf life compared to other encapsulation processes because the atmospheric gases diffuse slowly providing a nearly impermeable barrier to oxygen in the surrounding environment. For example, spray-drying produces a shelf life of approximately one year while the extrusion process produces a shelf life of five years. The current process being used was developed by Quellet et al. (2001) and includes a lower heat process using a mass of potato starch, glycerol and water. These ingredients are gelatinized using a twin-screw extruder at 100°C. The ingredients are then cooled and the core material is injected at a temperature of 50°C where ropes are extruded, dried and divided into pieces. This technique reduces flashing of volatile flavors which would be a better alternative for sensitive flavors. The microcapsule size tends to be larger with this process (500-1000um) which is considered a limitation for palatability reasons (Gouin, 2004).

Liposome Microencapsulation

Liposomes are structures comprised of lipid bilayers that contain aqueous or liquid compartments. Liposomes are utilized as model membranes and drug delivery systems. An advantage to this process is the stability liposomes provide to water-soluble material in high water activity applications. It also allows for targeted delivery of core material to specific parts and has been used extensively in the food industry. For example, the controlled release of proteinases has been used in dairy products to improve the flavor of cheddar cheese (Kheadr et al., 2000). The process of liposome microencapsulation can also be beneficial for decreasing vapor pressure in the water activity of food without decreasing the moisture content. "There are two principal requirements for liposome microencapsulation: the lipid must have a negative ΔG value for bilayer structure formation and sufficient energy must be put into the system to overcome the energy barrier" (Kim et al., 1991). Before the microencapsulation process begins, liposome preparation must occur. The three different methods for preparation are micro-emulsification, ultrasonication and membrane extrusion. Liposomes that are prepared through micro-emulsification are put through a micro-fluidizer. This machine pumps at a very high pressure (10,000 psi) to reduce the size of the liposome. Membrane extrusion utilizes dispersion during disruption of the lipid bilayer and passes through a polycarbonate membrane. There are various techniques that can be utilized to create a liposome microcapsule including electron microscopy, radiotracers, fluorescence quenching, ultrasonic absorption, electron spin resonance spectroscopy, and nuclear magnetic resonance spectroscopy. Each technique has advantages and disadvantages.

Conclusion

Since nitrogen excretion and ammonia runoff can lead to detrimental agroecosystem effects and eventually become a human health concern (Cowling and Galloway, 2002; Hristov, 2011), improving nitrogen efficiency while maintaining milk production is essential in the dairy industry. In order to upkeep milk production and quality, amino acid requirements of high producing animals must be met without overfeeding CP leading to excess nitrogen waste. The amino acid requirements of lactating dairy cows are dependent on diet. Lysine and methionine are often recognized as the first limiting AAs for milk production (Polan et al., 1991), but it is also seen that histidine and arginine can impact effectiveness of an essential AA profile (Lee et al., 2012; Ding et al., 2019).

Methods to supplement the first limiting Aas include rumen degradation protection and the process of urea recycling to improve nitrogen efficiency, meet protein requirements and reduce nitrogen excretion. In fact, supplementation of RDP can be used to support urea recycling for cattle eating low quality forages and improve forage utilization (Köster, 1996) and animal performance (Mathis et al., 1999). Additionally, free amino acids can undergo a rumen-protection method to successfully by-pass rumen degradability and be absorbed. Heat or chemical treatment, microencapsulation, AA analogs, and esophageal groove closures are examples of different methods of rumen protection. Microencapsulation is the most commonly used technique in the human and livestock food industry which encloses micron-sized particles in a shell to protect core material from degradation (Jyothi et al., 2010). Thus, with the use of a protection method, individual amino acids can be delivered and absorbed by the cow to maintain milk production and improve milk components. The addition of amino

acids being supplied to the diet will allow for a reduction of crude protein levels in diet. This reduction could potentially decrease feed costs, improve nitrogen efficiency and reduce nitrogen excretion into the environment (Rogers et al., 1987; Leonardi et al., 2003).

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CHAPTER 2: LACTATION PERFORMANCE AND NITROGEN EFFICIENCY OF DAIRY COWS FED INCREASING AMOUNTS OF MICROENCAPSULATED METHIONINE

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Abstract

Amino acid requirements of high-producing dairy cows may be greater than what dietary and microbial crude protein can provide. Therefore, study objectives were to determine lactation performance of dairy cows fed rumen-protected methionine (**MET**; Timet®, VETAGRO, Italy). Individually-fed, multiparous Holstein cows (n = 48, 127 ± 41 DIM and 671 ± 8 kg BW) were used in a replicated 4 × 4 Latin square design with 28-d periods. Dietary treatments consisted of a basal TMR deficient in metabolizable MET (-10 g/cow/day): 1) Control (**CON**) with no supplement; 2) low methionine (**LM**) diet with 11 g/cow/d MET; 3) medium methionine (**MM**) containing 19.25 g/cow/d MET; and 4) high methionine (**HM**) diet plus 27.5 g/cow/d MET. Milk yield and feed intake data from the last 7 d of each period were used for analyses; BW, BCS and milk components were determined on d 27 and 28 of

each period. Statistical analyses were performed using the MIXED procedures of SAS with square, period within square, and treatment as fixed effects and cow within square as a random effect. Linear, quadratic and cubic effects were also tested. No evidence of quadric or cubic effects was observed for any of the response variables. Dry matter intake was similar ($P = 0.64$) across treatments whereas milk production increased linearly from 38.0 ± 0.87 kg/d for CON to 39.7 ± 0.87 kg/d for HM. Milk fat concentration was similar across treatments ($P = 0.83$) averaging 3.69 ± 0.88% while milk protein concentration tended to increase linearly ($P = 0.11$) from 3.17 ± 0.04% for CON to 3.21 ± 0.04% with high MET supplementation. Overall, supplementation with MET resulted in greater ($P \leq 0.01$) yield of milk protein and fat. Consequently, yield of energy-corrected milk (**ECM**) increased linearly; cows consuming the CON diet produced 38.3 ± 1.05 kg ECM/d whereas MET supplementation resulted in 41.4, 40.9 and 41.7 ± 1.05 kg ECM/d, for LM, MM and HM, respectively. Concentration of MUN averaged 13.5 ± 0.23 mg/dL across treatments ($P = 0.58$). These data suggest that the MET supplement increased the supply of metabolizable methionine resulting in increased yield of milk and milk components.

Keywords: Limiting amino acids, nitrogen efficiency, rumen protected

Introduction

Dairy cattle and other ruminant animals utilize dietary and rumen microbial crude protein (MCP) as sources of amino acids (AA) (Aguirre-Villegas et al., 2017). Dietary protein is classified as either rumen undegraded protein (RUP) or rumen degradable protein (RDP); RUP will bypass rumen degradation and be digested in the small intestine while RDP is digested by microbes in the rumen and incorporated into microbial protein which eventually moved to and is digested in the small intestine. Higher producing dairy cattle

have an AA requirement that is often greater than what the diet and MCP are able to provide, especially for lysine and methionine (Met), when corn and soybean-based diets are fed. Supplementation with essential AA is often used as a strategy to meet these greater requirements, although microbial degradation of free AA in the rumen impairs the response to this strategy. Alternatively, producers often overfeed RUP in an attempt to meet the essential AA requirements. This strategy is wasteful, expensive and leads to inefficient nitrogen utilization. The excess nitrogen resulting from overfeeding protein is ultimately metabolized to urea, which is an energetically costly process and can lead to reduced cow body condition scores. The increased weight loss can be offset by increasing fat content in the diet; however, this is an increased cost to producers. In addition, the excess nitrogen can also have detrimental impacts on the environment. The nitrogen excreted in dairy manure can lead to nitrous oxide and ammonia production in livestock housing, in manure storage facilities, and following application to the land. Nitrous oxide adds to eutrophication through run-offs and can adversely impact crop growth if over-applied to soils (Johnson et al., 2016). To avoid the consequences of overfeeding protein, and since dietary protein and MCP are insufficient for higher producing dairy cattle, rumen-protection strategies can be employed to provide a more stable supply of these essential AA. Microencapsulation is a rumen-protection process that coats a nutrient or molecule with a matrix that protects the core material from microbial degradation (Jyothi et al., 2010). Methionine is considered as one of most limiting amino acid in dairy cattle in North America; Timet ® (VETAGRO, Italy) provides a source of DL-Met microencapsulated within a lipid matrix. Thus, this study's objectives were to feed increasing amounts of microencapsulated methionine and evaluate effects on lactation performance and nitrogen efficiency. It is postulated that this rumen

protected source of MET may supply the necessary metabolizable Met needed by high-producing cows. Additionally, diet formulations with rumen protected AA would allow those diets to have lower total crude protein, potentially increasing the efficiency of nitrogen use and reducing nitrogen excretion into the environment (Rogers et al., 1987; Leonardi et al., 2003).

Materials and Methods

Animals, Experimental Design, and Treatments

All procedures were approved by the Institutional Animal Care and Use Committee of Iowa State University (Ames, IA). Forty-eight multiparous Holstein cows (127 ± 41 DIM and 671 ± 8 kg BW; mean ± SD) were used in replicated 4 × 4 Latin square design. In each 28-d period, cows within a square were randomly assigned to 1 of 4 dietary treatments: 1) Control (**CON**) containing 100 g ground corn with no supplement; 2) low methionine (**LM**) diet with 11 g/cow/d MET; 3) medium methionine (**MM**) containing 19.25 g/cow/d MET; and 4) high methionine (**HM**) diet plus 27.5 g/cow/d MET. Each treatment was individually top-dressed on a basal TMR (Table 1) deficient in metabolizable MET (-10 g/cow/day).

Cows were housed in a free-stall barn equipped with individual feeding gates (Calan Broadbent Feeding System; American Calan, Northwood, NH). Daily care involved milking at 4:00, 12:00, and 20:00 h. Cows were individually fed a TMR at 110% ad libitum intake which was delivered once daily (700 h). Feed allowance and refusals were measured and recorded daily.

Sampling and Data Collection

Feed analyses. Samples of the basal TMR were collected on d 27 and 28 of each period and pooled to obtain a composite sample by period. Feed samples were placed in a forced-air

oven at 65°C for 48 h to determine DM, subsequently ground (1-mm screen; Wiley Mill, Arthur H. Thomas Co.; Philadelphia, PA) and stored at room temperature. The diet was analyzed for nutrient composition by an external laboratory (Cumberland Valley Analytical Services; Waynesboro, PA). Analyses included DM (method 930.15; AOAC International, 2000), starch (Hall, 2009), NDF (Van Soest et al., 1991), ADF (Van Soest et al., 1991) and ash (method 942.05; AOAC International, 2000). Determination of corn silage DM was performed once weekly and the TMR was adjusted accordingly. Total Kjeldahl Nitrogen Digestion of feed samples was used to determine the sum of organic and ammonia nitrogen.

Fecal and urine analyses. Indigestible neutral detergent fiber (iNDF) was used as an internal marker to estimate fecal output to determine apparent total tract digestibility based on fecal samples collected on d 27 and 28 of each period. Fecal samples were dried and ground as previously described for feed samples. Dry and ground fecal samples from each cow were analyzed for DM (method 930.15; AOAC International, 2000). Determination of iNDF was performed in quadruplicate by incubating 5 × 10 cm Dacron bags containing 1.25 g of sample material, either TMR or fecal, in two rumen-cannulated, lactating cows for 288 h (Huhtanen et al., 1994). The bags were then retrieved from the rumen, washed, dried, and analyzed for NDF (Van Soest et al., 1991). Urine samples were collected on d 27 and 28 of each period and stored frozen at -20°C before being submitted to the Department of Veterinary Pathology of Iowa State University for determination of creatinine analysis. This metabolite was used as an internal marker to estimate urinary output. Fecal and urine samples were also analyzed for nitrogen content using Total Kjeldahl Nitrogen Digestion (Leco FP528, St. Joseph, MI). Fecal samples were ground through a 1mm screen and 0.1 ± 0.005 g samples were used to perform nitrogen digestion. Urine samples were thawed and 1mL was pipetted for nitrogen analysis.

Milk data collection. Individual milk production was measured and recorded daily utilizing SmartDairy. Milk weights from the last 7 d of each period were used to evaluate milk production. Individual milk samples for milk composition analysis were obtained at 6 consecutive milking shifts on day 27 and 28 of each period. After collection, the 6 individual samples were composited. Samples were stored at 4°C with a preservative (Bronopol tablet; D & F Control System, San Ramon, CA) until analysis. Milk samples were analyzed for protein, fat, lactose, MUN, and somatic cell count at an external laboratory (Dairy Lab Services, Dubuque, IA), using infrared analysis equipment and approved procedures (AOAC International, 1995; method 972-16). Yields of milk components were estimated according to milk weight and time of collection.

Plasma analyses. Coccygeal samples were collected from all 48 cows after each milking shift on d 27 and 28 of each period. Subcutaneous abdominal vein samples were collected from 8 cows with highest milk yield and fewest DIM following each milking shift as well. All samples were collected using 10 mL vacuum tubes with K_2EDTA (BD, Franklin Lakes, NJ). Plasma was harvested after centrifugation at 1500 × *g* for 15 min at 4°C and the plasma was subsequently frozen at -20°C until analysis. The coccygeal and subcutaneous abdominal vein samples from the 8 selected cows were then thawed and composited to give a representation from each period. Composited samples were then refrozen and sent off for analysis. For amino acid analyses, samples were analyzed at the Experiment Station Chemical Laboratories of the University of Missouri-Columbia.

Animal measurements. Body weight and BCS (1 to 5 scale) were measured on d 27 and 28 of each period after milking. The scoring method used was similar to that of Wildman

et al. (1982) but reported to the quarter point. Two evaluators assessed BCS independently on 2 consecutive days of each period and scores were averaged.

Calculations and Statistical Analysis

Treatments were provided for all 28 d of each period and data collection was performed on d 27 and 28. All milk yield (MY) and DMI measurements were composited to means of the last 7 and 2 d of each period for analyses.

Fat-corrected milk (FCM) was calculated as described by Tyrrell and Reid (1965) using the following equation: 3.5% FCM = (0.432 × milk yield, kg) + (16.23 × milk fat yield, kg). Extraction efficiency of AA of the mammary gland was calculated as: arterial AA plasma – venous / arterial concentration × 100. Feed efficiency (FE) was calculated as: FE = MY ÷ DMI. Dry matter digestibility was calculated as: DIG % = [100 - (100 × iNDF TMR% ÷ iNDF Fecal %)]. Urine output (L/d) was calculated using estimations from Whittet (2004) assuming internal creatinine averages of 28 mg/kg of BW. The excretion of nitrogen in the feces was calculated from the obtained nitrogen digestibility and intake.

Data were analyzed as a replicated 4 × 4 Latin square design using the MIXED procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC.). Fixed effects of the model were the square, period within the square and treatment. Cow within the square was the random effect of the model. The linear model for these data is as follows:

$$y_{ijkm} = \mu + \tau_m + \beta(\tau)_{im} + \rho(\tau)_{jm} + \alpha_k + \varepsilon_{ijkm}$$

Where y_{ijkm} is the response variable of the i_{th} cow in m_{th} square during the j_{th} period consuming the k_{th} treatment, ; μ is the overall mean; τ_m depicts the fixed effect square m; $\beta(\tau)_{im}$ is the random effect of cow i within square m; $\rho(\tau)_{jm}$ depicts the fixed effect of period j within square m; and α_k is the fixed effect of treatment. The error term ε_{ijkm} was assumed to be normally and independently distributed, with variance σ_e^2. Statistical significance for all treatments was declared at $P \leq 0.05$; tendencies were assumed at $P \leq 0.10$. Results are presented as least square means ± the largest standard error of the mean.

Results

Lactation Performance

Dry matter intake did not differ ($P = 0.39$) among dietary treatments averaging 23.33 kg/d ± 0.46 (Table 2). Milk yield increased linearly with increasing levels of Met supplementation. Milk yield increased from 38.05 kg/d ± 0.87 with no supplemental Met to 39.73 ± 0.86 with the high Met treatment ($P = 0.03$, Table 2). Overall, milk fat ($P = 0.42$) and lactose concentration ($P = 0.37$) were similar across all treatments. Similarly, milk urea nitrogen ($P = 0.41$) and somatic cell count ($P = 0.73$) were not different between treatments. Milk protein concentration tended to increase linearly ($P = 0.11$) from 3.17 ± 0.04% for CON to 3.21 ± 0.04% with high MET supplementation. When milk yield was taken into account, milk protein and fat were greater with Met supplementation ($P \leq 0.01$, Table 2). Fat corrected milk increased for cows on treatments LM, MM, and HM when compared to CON ($P = 0.01$, Table 2). Similarly, there was a treatment and linear effect observed for energy corrected milk production ($P \leq 0.01$, Table 2).

Digestibility, Urine and Fecal Output

There were no differences (P = 0.23) for DM digestibility averaging 59.01 ± 2.65%. Similarly, urine output (P = 0.38, 6.21 ± 0.79 L/d) and fecal output (P = 0.59, 10.36 ± 1.03 kg/d) were not different across all dietary treatments

Nitrogen Analyses

No significant differences were observed for intake N (P = 0.45), urinary N (P = 0.55), or milk N (P = 0.51) g/d. There was a tendency for a linear decrease of fecal mass of nitrogen with increasing amounts of microencapsulated methionine fed (P = 0.05, Table 6). Digested nitrogen (g/d) was significantly increased across all treatments when compared to cows fed the control diet (P = 0.003). Similarly, retained nitrogen linearly increased across all treatments (P = 0.01) (mean values of 524.37 ± 61.33 g/d) when compared to cows fed the control diet. Retained nitrogen was calculated by taking the difference of milk and manure N from intake N. Productive nitrogen (g/d) was significantly different across all treatments (P = 0.02, Table 3) compared to cows fed the control diet and was calculated by adding milk N and retained N. Percentage of intake N was then calculated and there were significant differences observed for fecal N (P = 0.02), manure N (P = 0.02), retained N (P = 0.04) and productive N (P = 0.02).

Plasma Chemistry Profile

Arterial plasma concentrations of methionine and tryptophan increased with increasing Met supplementation ($P \leq 0.01$), although there were no differences in the arterio-venous concentrations (Table 4). Extraction efficiencies of methionine, lysine, threonine, tryptophan, histidine and arginine were all significantly different across treatments ($P \leq 0.01$, Table 5).

Discussion

The objective of this study was to evaluate the effect of supplementing increasing amounts of microencapsulated methionine on lactation performance and nitrogen efficiency. We postulated that dietary supplementation of Met would enhance milk yield, milk components, and nitrogen efficiency. It is important to note that during this trial, a mastitis outbreak resulted in missing data points from MM treatments in period 2. This may have contributed to the slight decrease in production parameters between LM and MM treated animals.

Methionine treatments were supplemented to a formulated basal diet deficient in metabolizable Met (10g/cow/day) to meet the AA requirement of the cow and to subsequently reduce nitrogen excretion. However, CON and LM treatments were still considered to be deficient in metabolizable methionine. The MM treatment was formulated to meet the AA requirement of a lactating dairy cow and HM treatment provided excess methionine. In this study, a linear increase of milk yield was observed, while there was no effect of treatments on DMI. Although there was no difference in milk protein concentration with Met supplementation, milk protein yield increased from CON (1.16 kg) to HM (1.27 kg). By contrast, Leonardi and others (2003) reported that supplementation of methionine with two different levels of crude protein in basal diet (16.1% vs 18.8%) increased the concentration of milk protein, but no differences in milk protein yield were observed. Those workers also observed no differences in milk yield. It is important to note, however, that in the present study the true crude protein content of the basal diet was higher than expected (17.3% vs 16.0%) following feed analysis. This difference may have contributed to the tendency of a linear increase in milk yield.

Extraction efficiency is a calculated term that describes how much of a circulating AA is taken up by the mammary gland. With increasing amounts of Met supplied there was a decrease in AA extraction efficiency within the mammary gland. This decrease was also observed for methionine, lysine, threonine, tryptophan, histidine and arginine (Table 5). The NRC postabsorptive model predicts that the gross efficiency conversion of absorbed protein to milk protein will increase as the protein supply increases or until requirement for milk protein is met. Once milk protein requirements are exceeded, extraction efficiency will decline (NRC, 1985). The decrease in extraction efficiencies seen in this study support this model, demonstrating that there was an improvement in absorptive efficiency of AA's once a threshold level of supplementation was reached. Because the HM treatment still resulted in some level of increased absorptive efficiency, the amount of Met supplemented did not surpass the requirement needed for milk protein as expected.

We also anticipated that the decrease in crude protein and increase in supplemented methionine would improve nitrogen efficiency and decrease excretion. Nitrogen excretion from dairy cattle is a precursor for nitrous oxide and ammonia. Nitrous oxide plays a major role in greenhouse gas emissions and the reduction of nitrogen output from dairy facilities has the potential to reduce nitrous oxide from entering the atmosphere (Johnson et al., 2016). Beef and dairy operations contribute 41 and 20% of total livestock emissions, respectively (Gerber et al., 2013). Manure N can also contribute to eutrophication of surface waters (McCubbin et al., 2002; Diaz and Rosenberg, 2008). Manure with large amounts of ammonia can lead to overfertilization of soil leading to damaged crop growth and formation of particulate matter when combined with nitrates and sulfates (Fowler et al., 2013). Human health problems can arise from these nitrogen compounds being released into the

environment, including lung disease, chronic bronchitis and premature mortality (McCubbin et al., 2002; Fowler et al., 2013). In this study milk and urine nitrogen excretion were not different across treatments. This may have been affected by measured CP being higher than our planned formulations. However, there was a 29.2% decrease in fecal nitrogen from CON to HM treatments. Fecal nitrogen includes undigested dietary, microbial, and endogenous N (A-A). Therefore, in agreement with improved absorption in the mammary gland, the more Met supplemented increased GI tract absorption ability and may have resulted in an improvement regarding nitrogen efficiency. The reduction in amount of fecal N excreted per cow can be beneficial in mitigating negative human health and agroecosystem effects.

Conclusion

Providing additional metabolizable methionine in diets of high-producing cows can benefit the producer and the cow. Supplementation of this essential amino acid will reduce overfeeding of crude protein and minimize nitrogen excretion. When increasing amounts of Met were fed to cows there was a corresponding increase in plasma methionine. That led to increased production performance. Milk protein yield increased by 6.9%, 7.8% and 9.5% for LM, MM, and HM when compared to CON, respectively. Similarly, milk fat yield increased by 7.4%, 5.9% and 7.4% for LM, MM and HM. Met supplementation also caused energy corrected milk to increase linearly across treatments. Greenhouse gas emissions are a concern across the industry and reducing the amount of nitrogen excreted can greatly reduce this concern. Dairy cows fed increasing amounts of microencapsulated methionine linearly decreased the amount of fecal nitrogen excreted by 19.5%, 20.8%, and 29.2% for LM, MM, and HM when compared to CON, respectively.

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Table 1. Ingredient and analyzed chemical composition of basal diet[1]

Dietary ingredients	% of dietary DM
Corn silage	40.29
Alfalfa hay	15.50
Ground corn	15.32
Soybean meal	9.30
Cottonseed	5.68
Quality Liquid Feed (Molasses)	2.93
Grain mix[2]	10.98
Amino Acids	
Met Met	0.18
Met Lys	0.74
Nutrient analysis, % DM	
CP	17.30
NDF	31.80
ADF	21.50
Starch	23.70
Ash	8.10

[1] Values represent an average of composite samples collected throughout the trial (n = 4)

[2] Contained ground corn, soybean meal, DDG, bloodmeal, soybean hulls, pork meat and bone meal, sodium bicarb, calcium carbonate, choice white grease, salt, urea, vitamin mix, magnesium oxide, monocalcium phosphate, monensin, biotin and chelated minerals

Table 2. Performance on dairy cows consuming microencapsulated methionine

	Dietary Treatment[1]				SEM[2]	*P*-value[3]			
	CON	LM	MM	HM		Trt	Lin	Quad	Cubic
Dry matter intake, kg/day	23.59	23.39	23.02	23.33	0.46	0.64	0.39	0.49	0.48
Milk yield, kg/day	38.05	39.31	39.16	39.73	0.87	0.11	0.03	0.53	0.41
3.5% FCM[4]	37.77^{b}	40.88^{a}	40.15^{a}	40.90^{a}	1.08	0.01	0.007	0.13	0.17
ECM[5]	38.35^{b}	41.45^{a}	40.90^{a}	41.67^{a}	1.05	0.009	0.004	0.14	0.21
Protein, %	3.17	3.16	3.22	3.21	0.04	0.18	0.11	0.66	0.15
Protein, kg	1.16^{b}	1.24^{a}	1.25^{a}	1.27^{a}	0.03	0.003	0.0006	0.23	0.36
Fat, %	3.72	3.69	3.70	3.66	0.88	0.83	0.42	0.87	0.67
Fat, kg	1.35^{b}	1.45^{a}	1.43^{a}	1.45^{a}	0.05	0.03	0.02	0.14	0.25
Lactose, %	4.66	4.63	4.67	4.62	0.03	0.35	0.37	0.69	0.13
Lactose, kg	1.71^{b}	1.85^{a}	1.82^{a}	1.86^{a}	0.05	0.02	0.009	0.17	0.24
MUN, mg/dL	13.58	13.55	13.71	13.32	0.23	0.58	0.41	0.38	0.49
Somatic Cell Count	281.42	394.01	222.94	388.52	149.48	0.58	0.73	0.87	0.18
Body weight, kg	672.82	671.48	670.03	671.54	8.51	0.82	0.54	0.53	0.69
Body condition score[6]	3.34	3.33	3.32	3.32	0.03	0.83	0.40	0.69	0.99

[1]**CON** = Control (100g corn); **LM** = Low Methionine (80g corn and 20g Timet); **MM** = Medium Methionine (65g corn and 35g Timet); **HM** = High Methionine (50g corn and 50g Timet).
[2]Highest standard error of treatment mean is shown.
[3]Main effect of treatment.
[4]3.5% Fat corrected milk = [milk fat (kg) × 16.216] + [milk yield (kg) × 0.4324].
[5]ECM = [0.327 × milk yield (kg)] + [12.95 × milk fat (kg)] + [7.65 × milk protein (kg)].
[6]Body condition scores 1-5 scale (Wildman et al., 1982).
[a-d]Values in the same row with different superscript differ significantly.

Table 3. Arterial plasma concentration of essential amino acids on dairy cows consuming microencapsulated methionine

	Dietary Treatment[1]				SEM[2]	*P*-value[3]			
	CON	LM	MM	HM		Trt	Lin	Quad	Cubic
AA[4], µg/mL									
Methionine	**2.83$_{c}$**	**3.23$_{b}$**	**3.35$_{b}$**	**3.78$_{a}$**	0.12	< 0.01	<.0001	0.65	0.29
Lysine	11.55	11.98	12.34	13.13	0.84	0.23	0.05	0.61	0.85
Phenylalanine	8.40	8.27	7.75	8.34	0.52	0.26	0.53	0.22	0.18
Valine	36.26	36.52	35.05	36.35	2.51	0.73	0.81	0.69	0.35
Threonine	14.67	14.35	15.01	15.69	1.17	0.14	0.06	0.14	0.63
Tryptophan	6.82$_{b}$	7.29$_{a}$	7.39$_{a}$	7.62$_{a}$	0.41	< 0.01	< 0.01	0.51	0.55
Isoleucine	16.51	17.15	15.86	16.81	1.30	0.49	0.91	0.91	0.15
Histidine	7.79	8.27	7.95	8.73	0.35	0.20	0.09	0.62	0.19
Arginine	13.26	13.43	14.29	14.93	0.81	0.12	0.03	0.48	0.68
Leucine	26.02	25.63	24.02	25.29	2.16	0.47	0.40	0.48	0.33

[1]**CON** = Control (100g corn); **LM** = Low Methionine (80g corn and 20g Timet); **MM** = Medium Methionine (65g corn and 35g Timet); **HM** = High Methionine (50g corn and 50g Timet).
[2]Highest standard error of treatment mean is shown.
[3]Main effect of treatment.
[4]Amino acid.
[a-d]Values in the same row with different superscript differ significantly.

Table 4. Arterio-venous differences of essential amino acids on dairy cows consuming microencapsulated methionine

	Dietary Treatment[1]				SEM[2]	*P*-value[3]			
	CON	LM	MM	HM		Trt	Lin	Quad	Cubic
AA[4], µg/mL									
Methionine	1.92	1.71	1.74	1.73	0.12	0.45	0.24	0.38	0.65
Lysine	7.21	6.66	6.63	6.68	0.52	0.66	0.36	0.48	0.89
Phenylalanine	3.72	3.26	3.22	3.28	0.25	0.29	0.16	0.27	0.85
Valine	8.11	6.54	5.94	6.23	0.68	0.08	0.03	0.19	0.88
Threonine	3.95	3.49	3.38	3.28	0.33	0.39	0.13	0.63	0.88
Tryptophan	0.86	0.86	0.63	0.61	0.12	0.09	0.03	0.70	0.27
Isoleucine	6.06	5.08	4.82	5.13	0.54	0.22	0.13	0.20	0.92
Histidine	2.10	1.84	1.75	1.67	0.16	0.23	0.06	0.64	0.87
Arginine	6.22	5.76	5.26	5.74	0.49	0.33	0.25	0.27	0.47
Leucine	9.76	8.08	7.81	8.18	0.81	0.17	0.10	0.17	0.94

[1]**CON** = Control (100g corn); **LM** = Low Methionine (80g corn and 20g Timet); **MM** = Medium Methionine (65g corn and 35g Timet); **HM** = High Methionine (50g corn and 50g Timet).
[2]Highest standard error of treatment mean is shown.
[3]Main effect of treatment.
[4]Amino Acid.

Table 5. Extraction efficiency[1] of amino acids of mammary gland on dairy cows consuming microencapsulated methionine

	Dietary Treatment[2]				SEM[3]	*P*-value[4]			
	CON	LM	MM	HM		Trt	Lin	Quad	Cubic
AA[5], µg/mL									
Methionine	67.76[a]	53.14[b]	51.58[b]	45.87[c]	2.77	<0.001	<.0001	0.12	0.21
Lysine	65.52[a]	56.82[ab]	53.77[b]	52.06[b]	3.24	0.04	0.007	0.52	0.97
Phenylalanine	45.69	40.12	41.60	40.89	3.70	0.24	0.14	0.23	0.39
Valine	22.33	19.06	17.49	18.25	1.69	0.06	0.03	0.16	0.76
Threonine	27.87[a]	22.42[ab]	21.02[b]	19.06[b]	2.19	0.04	0.008	0.49	0.73
Tryptophan	12.98[a]	11.88[ab]	9.10[b]	7.83[cb]	1.85	0.03	0.005	0.70	0.51
Isoleucine	36.84	31.66	31.15	32.56	3.01	0.28	0.19	0.19	0.92
Histidine	27.26[a]	22.31[ab]	22.29[b]	19.30[b]	2.04	0.05	0.01	0.66	0.41
Arginine	47.32[a]	43.73[ac]	37.06[cb]	39.13[c]	3.64	0.02	0.007	0.32	0.18
Leucine	38.25	33.68	33.94	34.79	3.34	0.49	0.32	0.28	0.80

[1]Extraction efficiency = arterial – venous / arterial concentration × 100

[2]**CON** = Control (100g corn); **LM** = Low Methionine (80g corn and 20g Timet); **MM** = Medium Methionine (65g corn and 35g Timet); **HM** = High Methionine (50g corn and 50g Timet).

[3]Highest standard error of treatment mean is shown.

[4]Main effect of treatment.

[5]Amino Acid.

[a-d]Values in the same row with different superscript differ significantly.

Table 6. Effects of feeding increasing amounts of microencapsulated methionine to dairy cattle on N metabolism

	Dietary Treatment[1]				SEM[2]	*P*-value[3]			
	CON	LM	MM	HM		Trt	Lin	Quad	Cubic
Mass (g/d)									
Intake N	1200.97	1166.61	1170.83	1252.07	52.69	0.45	0.46	0.77	0.17
Fecal N	635.79	511.98	503.60	449.98	75.92	0.21	0.05	0.63	0.64
Digested N	565.19	655.51	667.16	803.45	51.66	0.02	0.003	0.49	0.35
Urinary N	54.78	63.94	57.81	58.94	4.47	0.25	0.55	0.16	0.16
Manure N	690.57	576.10	561.09	509.34	78.38	0.25	0.06	0.68	0.71
Milk N[4]	197.97	194.23	215.31	213.19	19.12	0.51	0.25	0.81	0.39
N Retained[5]	312.43	390.52	412.29	524.37	61.33	0.09	0.01	0.64	0.59
Productive N[6]	510.40	590.84	609.58	743.30	52.45	0.02	0.004	0.44	0.42
% N Intake									
Fecal N	52.13	45.76	45.08	38.06	4.59	0.11	0.02	0.83	0.51
Urine N	4.68	5.53	5.25	4.94	0.31	0.06	0.46	0.01	0.39
Manure N	56.82	51.32	50.32	43.05	4.66	0.12	0.02	0.71	0.55
Milk N	18.03	17.36	19.50	18.09	1.65	0.52	0.63	0.84	0.19
N Retained	25.15	30.86	32.46	38.52	5.11	0.21	0.04	0.87	0.71
Productive N	43.18	48.67	49.67	56.94	4.66	0.12	0.02	0.71	0.55

[1]**CON** = Control (100g corn); **LM** = Low Methionine (80g corn and 20g Timet); **MM** = Medium Methionine (65g corn and 35g Timet); **HM** = High Methionine (50g corn and 50g Timet).
[2]Highest standard error of treatment mean is shown.
[3]Main effect of treatment.
[4]Milk N yield (kg) per kg N intake × 100
[5]Retained N = intake N – (manure N + milk N)
[6]Productive N = milk N + retained N

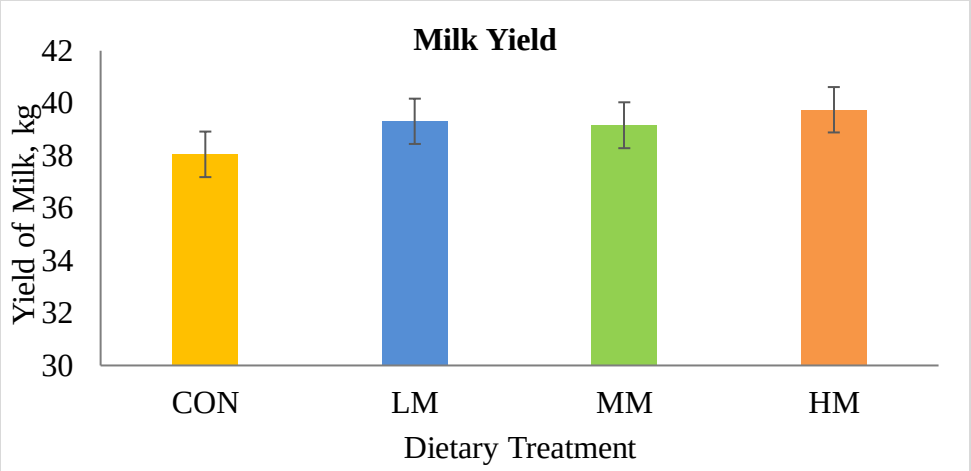

Figure 1: Effects of feeding microencapsulated methionine on milk yield in lactating dairy cows.

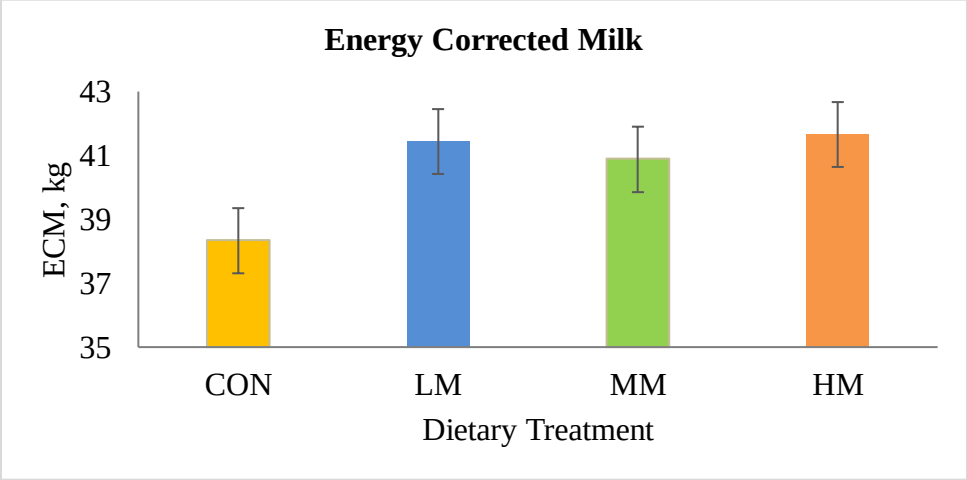

Figure 2: Effects of feeding microencapsulated methionine on energy corrected milk in dairy cows

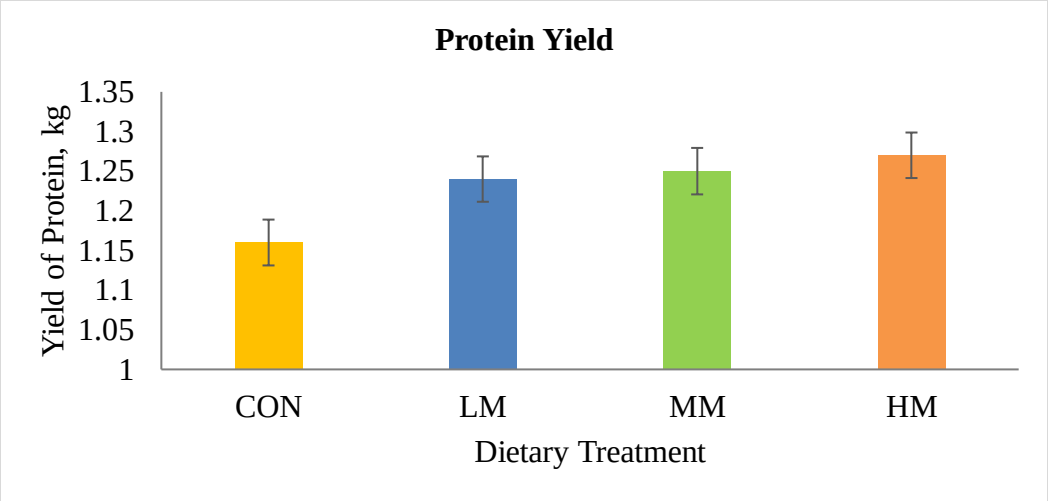

Figure 3: Effects of feeding microencapsulated methionine on milk protein yield in lactating dairy cows.

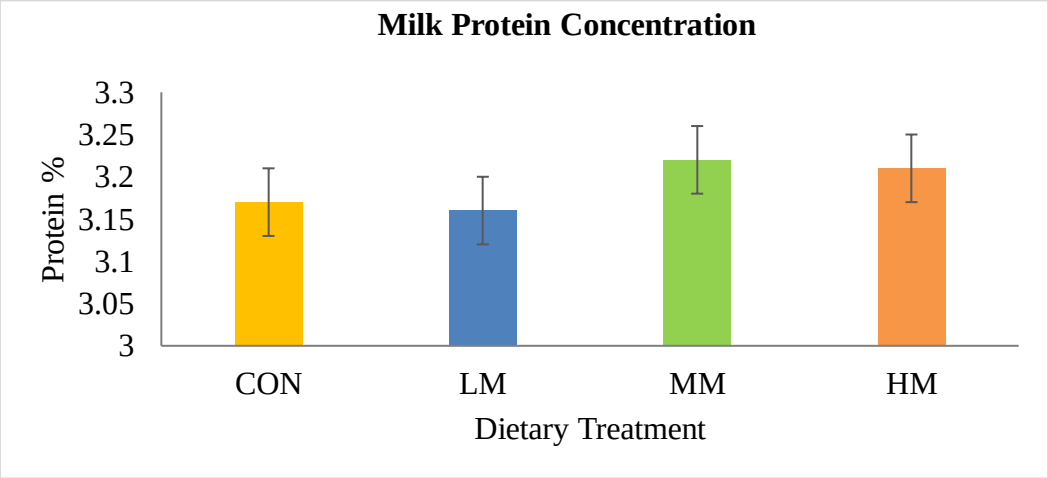

Figure 4: Effects of feeding microencapsulated methionine on milk protein concentration in dairy cows.

CHAPTER 3: SUMMARY AND IMPLICATIONS

Over the past few decades, a significant amount of research has focused on reducing negative environmental effects of the dairy industry. Balancing dairy cattle diets to reduce negative environmental effects, meet nutritional requirements, and maintain high production can pose a challenge. Since the mammary gland exports vast amounts of milk protein, it has a requirement for both nonessential and essential amino acids (EAA) to support protein synthesis (Stelwagen, 2016). Diets are balanced making adjustments to support the first essential amino acid to become deficient (first limiting AA). Lysine and methionine are often labeled as the first liming AA for milk production in dairy cattle, depending on the diet (Polan et al., 1991). However, arginine and histidine also may have positive effects on milk production (Wang et al., 2014; Lee et al., 2012). Ruminant animals receive their amino acids (AA) through dietary protein and microbial crude protein (MCP). Dietary protein can be classified as either rumen-degradable protein (RDP) or rumen-undegradable protein (RUP). RDP is rapidly solubilized in the rumen producing ammonia for microbial use or broken down to free amino acids. The free AA join together to form peptide chains (MCP) that are transported down the digestive tract along with RUP to either be absorbed by the small intestine or eventually be excreted. However, greater producing animals may require more AA than the diet and MCP are able to provide.

Normally, the NRC recommends dairy cattle diets consist of 17.5% to 19.0% crude protein to support higher milk production. Increasing crude protein in the diet is the most direct method and has been used in the past to increase milk yield (NRC, 2001). However, with this practice nitrogen excretion also increases resulting in increased ammonia emissions. Protein that has been consumed in a dairy cow is excreted in the form of urea nitrogen in

urine, feces or milk. Urea is then converted to ammonia through bacterial degradation by the enzyme urease. Depending on management and feed methods, 30% to 50% of N is released as ammonia (Hristov et al., 2009). Ammonia in the environment can lead to the formation of fine particulate matter, which can be a danger to human health by affecting the bronchioles and gas exchange inside of lungs (Hristov, 2011). Nitrogen can also be released as nitrous oxide after being excreted, which can be detrimental by contributing to eutrophication of run-offs and negatively impact agroecosystems (Johnson et al., 2016; Cowling and Galloway, 2002).

Thus, to minimize nitrogen excretion and maintain production, rumen-protected AAs are supplemented to bypass rumen degradability and improve protein utilization. Extensive research has been conducted supplementing the most common first limiting AA (Met) and evaluating effects on milk yield (Broderick et al., 2008; Schmidt et al., 1999), milk protein concentration (Zhao et al., 2019), milk protein yield (Lee et al., 2012) and nitrogen efficiency (Broderick et al., 2008). The majority of those earlier studies observed positive impacts on milk production parameters. However, Leonardi et al. (2003) saw no benefit and concluded that of Met was non-effective. Furthermore, additional studies have been conducted with varying diets and AA profiles and that data was reported as inconclusive (Aines et al., 2010; Ding et al., 2019; Paz et al., 2013). Although studies are contradicting, there is common ground in the belief that a diet containing a complete essential AA profile is more beneficial to the host animal than specifically selecting a single AA to supplement.

Various rumen-protection methods such as heat or chemical treatment, microencapsulation, AA analogs, and esophageal groove closure are available to supply these bypass AA's. Microencapsulation is the process of enclosing micron-sized particles in a

polymeric shell that protects the core material from microbial degradation (Jyothi et al., 2010). Microencapsulation has different techniques that are utilized depending on the desired outcome. Liposome microencapsulation is the most commonly used in the animal industry. An advantage to this method is the stability liposomes provide to water-soluble material in high activity situations. It can also target delivery of core material depending on number of layers applied (Gouin, 2004).

In the present study, the objectives were to feed increasing amounts of microencapsulated methionine (Met) and evaluate effects on lactation performance and nitrogen efficiency. We postulated that a low protein basal diet with supplemental Met would allow maintenance needs of the host animal to be met, increase milk yield, improve milk protein, and increase nitrogen efficiency. CON and LM treatments were formulated to be deficient in Met, MM met lactating animal's requirement, and HM was considered to be excess. However, no plateau when HM was supplemented was observed, which implies that HM treatment was still not exceeding the host animal's AA requirement. When increasing amounts of Met were fed to cows there was a corresponding increase in plasma methionine which led to an increase in production performance. A linear increase in milk yield was observed with a tendency to increase milk protein concentration. Furthermore, milk yield of fat and protein increased with supplementation. Amino acid absorption within the mammary gland was significantly improved with supplementation with Met. This response was not only observed in methionine, but supplementation enhanced the absorption for the majority of other essential AAs (Table 5). Regarding nitrogen (N), milk urea nitrogen (MUN) and urinary N were similar across all treatments. This may have been due to measured crude protein being higher than planned formulations. Digested and retained N as expected

increased linearly. Moreover, nitrogen excreted in feces was decreased with increasing amounts of Met. In agreement with previous studies, various milk components were enhanced, and fecal N excretion was reduced. Maintenance and high production were supported through the entire trial. Amino acid utilization was also enhanced with increasing amounts of methionine. Further research must be done to investigate if a lower CP diet than the present study's diet will have more substantial significance regarding milk production parameters and nitrogen efficiency.

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