RUMINAL ESCAPE AND INTESTINAL DIGESTIBILITY OF EXPERIMENTAL RUMINAL PROTECTED LYSINE SUPPLEMENTS

by

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(Under the Direction of John Bernard)

ABSTRACT

Ruminal escape (RE) and intestinal digestibility (ID) of experimental lysine supplements was evaluated. In trial one, no differences were observed in RE of DM and N of five products differing in source of hydrogenated oil (soybean oil [SB] or rapeseed oil [RS]) with 0 or 2% oleic acid in the RS coating. Intestinal digestibility of N was higher with RS than with SB and inclusion of oleic acid improved DM and N ID. In trial two, RE of N and lysine was greatest for two experimental products, but ID was highest for three commercial products. In trial three, RE of N and lysine decreased as oleic acid increased and as the concentration of lysine increased. No differences were observed in ID of N or lysine. These trials indicate that the proportion of lysine in the product and composition of the fat coating influences RE and ID of N and lysine.

INDEX WORDS: ruminal escape, intestinal digestibility

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DEDICATION

I would like to dedicate this paper to my parents, who have always given me support and encouragement. You always encourage me to hold on my dream and never give up. Thanks you all!

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CHAPTER 1

INTRODUCTION

Protein is one of the most critical limiting nutrition for maximizing yield of milk and milk protein in the diets of lactating cows. In ruminants, most of the dietary protein is degraded in the rumen to provide the amino acids and N precursors for the micro-organism to synthesize microbial protein. The excess N in the form of ammonia can be recycled back to rumen or excreted as urea in urine. 80% of the remaining fraction of dietary protein which escapes degradation in the rumen is digested in the small intestine. Sources of rumen undegradable protein (RUP) are frequently fed to improve the efficiency of metabolic protein utilization and to minimize that fraction of dietary protein degraded in the rumen and metabolized and excreted as urea.

Lysine and methionine are considered the most essential amino acids and co-limiting to meet requirements for milk synthesis (Wang et al. 2010). Socha et al. (2005) reported that supplementing ruminal protected methionine (RPMet) and ruminal protected lysine (RPLys) in the basal diets of early lactating cows could increase energy-corrected milk (ECM), milk true protein and milk fat, implicating that these are the top two most limiting essential amino acids. Various research has been conducted on improving ruminants productivity by feeding RPMet (Broderick et al. 2008; Rulquin et al. 2006). However, limited research has been conducted with RPLys because protected lysine products have not been available until recently.

Many techniques, such as heat treatment and formaldehyde treatment have been used to protect dietary proteins and coating the amino acids have been used to protect essential amino acids from degrading in the rumen. However coating essential amino acids is thought to be most

effective way to protect without detrimental effect to animal performance (Vallejo, 1996). A combination of unsaturated and saturated fatty acids have been used for coating. Jenkins and Jenny (1989) reported hydrogenated fatty acids had lower rumen digestibility and may be more useful to coat and protect amino acids from degradation as compared with other fats. Similar results have been reported by Pantoja et al. (1994), Harvatine and Allen (2006). Drackley et al. (2007) reported linear increases of total fatty acid digestibility with increasing oleic acid, which could be important for increasing intestinal digestibility of a coated lysine product. Less data are on the intestinal digestibility of the coating products, compared with the ruminal escape (Kamalak et al. 2005).

The objective of these research projects were to determine the effect of adding various percentage of oleic acid to a hydrogenated fat coating applied to experimental supplements containing different proportions of supplemental LYS on ruminal escape and intestinal digestibility of lysine employing the mobile bag technique and to evaluate the ruminal escape and intestinal lysine digestibility of several commercially available products.

CHAPTER 2

LITERATURE REVIEWED

CRUDE PROTEIN (CP)

Dietary protein is an essential part of an animal's diet to provide amino acids and N compounds for synthesis of protein tissues (muscle and connective tissue), enzymes, hormones, and other compounds essential for normal body function. In ruminants protein nutrition is complicated by the pregastric fermentation of the rumen. In contrast with monogastric animals, ruminants have a diverse population of rumen micro-organisms that both degrade dietary protein and synthesize microbial protein. This allows the ruminant to consume lower quality protein and convert it to higher quality protein. For lactating dairy cows, protein is one of the critical limiting nutrients influencing milk, and milk protein synthesis. Because higher producing dairy cows require larger quantities of protein to meet their requirements, higher quality protein that is resistant to microbial degradation is often fed to supplement microbial protein supplies.

The approximate protein content of feed ingredients is measured nutritionally as crude protein (CP). This is determined by measuring the N content in an ingredient and multiplying it by a factor of 6.25. The protein requirements of lactating dairy cattle were originally calculated using a factorial method (Mitchell, 1929; NRC, 1978) that was expressed as total CP (TCP). Protein requirements were calculated as using the following formula:

$$TCP=U+F+S+G+C/Ep +L/Ep$$

Where: TCP = total crude protein requirement in grams per day.

U = 6.25 x urine N

F = 6.25 x fecal metabolic N

S = protein lost in skin secretions, scurf and hair

G = protein deposited in or associated with the increase in body weight of growing cattle.

C = protein deposited in products of conception.

L = net protein required by synthesis of milk protein.

Ep = factors necessary to convert the sums of the net protein requirements to their equivalents in terms of dietary crude protein.

The subcommittee on N usage in ruminants (NRC, 1985) recommended absorbed protein as the preferred method for expressing protein requirements. Absorbed protein is defined as the digestible true protein which includes the ruminally synthesized microbial CP and dietary protein which escapes ruminal degradation. This method of calculating protein requirements was incorporated into the 7th edition of Nutrient Requirements of Dairy Cattle (NRC, 2001) but is referred to as metabolizable protein (MP).

THE ROLE OF PROTEIN IN NUTRITION

In the calf, Lassiter et al. (1963) reported that the digestibility coefficients for CP and crude fiber decreased when the crude protein content of the milk replacers decreased. These authors also noted that nitrogen retention decreases as the protein concentration in milk replacer decreased. Overall, calves grew normally with ADG of 0.8 lb, when fed milk replacer containing approximately 24% protein. When the protein concentration decreased from 24% to approximately 19%, growth rate of calves was not significantly altered. Blome et al. (2003)

reported that increasing CP in milk replacers from 16 to 26% increased average daily gain (ADG), disposition of structural and lean tissue and improved protein utilization. Bartlett et al. (2006) reported increased ADG and efficiency of gain when the protein to energy ratio in milk replacers was increased from 14% to 26% at 1.75 % of BW. Also, there's a relationship between dietary CP and its feeding rate on lean tissue gain. Gain of lean tissue is restricted when milk replacer was fed at 1.25 of body weight (BW) and contained 22% CP; however, when fed at 1.75% of BW additional gains in lean tissue occur with up to 26% CP.

In the lactating dairy cow, Law et al. (2009) reported that diets containing 173g of CP/kg of DM improved feed intake and animal performance in early lactation. Increasing dietary CP from 114 g of CP/kg to 173 g of CP/kg also increased plasma urea, albumin, and total protein concentrations. When diets containing 173g of CP/kg of DM are fed, increases in dry matter intake (DMI), milk yield, concentrations of plasma urea and total protein are observed early in lactation (up to d 150); however therefore, CP concentration can be reduced to 14.4% of dry matter (DM) without detrimental effects on animal performance. Spears et al. (1986) reported that supplementing protein at high concentration in winter increased calf gains and weaning weights, and the cows lost less BW.

McCormick et al. (1999) reported similar reproductive performance for cows fed ryegrass pasture in the morning and corn silage in the evening and supplemented with grain (moderate CP, moderate rumen-undegradable protein (RUP) control diet), compared with that of cows fed ryegrass pasture in the morning and corn silage in the evening and supplemented with a grain mix containing ground corn, soybean meal, corn gluten meal, and blood meal (moderate CP, high RUP). However, cows fed excess dietary CP had lower first service pregnancy rates and overall pregnancy rates. McCormick et al. (2001) reported that postpartum body condition loss

was greater for calving group cows fed a high CP supplement compared with control cows fed a moderate CP supplement. These results suggest that feeding excess supplemental RUP could increase days to first estrus and reduce first-service pregnancy rate of calving group cows. Results indicate that both excess postpartum body condition loss and inconsistent protein supplementation negatively affects days to first service and first-service pregnancy of grazing cows. Qureshi (2002) reported that cows fed lower CP intake displayed normal estrus compared with those cows that were anoestrus when fed higher CP. The dietary ratio of CP to metabolizable energy (CP: ME) in the normal cycling cows was narrow and constant, compared with a widely fluctuating anoestrus animals. The postpartum ovulation interval was correlated with ME intake, which was higher during estrous.

Different sources of dietary protein can affect the efficiency of its utilization in the animal. Piepenbrink (1998) reported that canola meal has lower rumen escape and resulted in lower absorption in the small intestine compared with blood meal, corn gluten meal, or menhaden fish meal. However, feeding canola meal supported greater microbial protein synthesis which has a more desirable balance of essential amino acids (EAA) than blood meal or corn gluten meal. Therefore, they recommend not to use blood meal and corn gluten meal as the only source of supplemental protein. Ipharraguerre and Clark (2005) reported that treated soy products, corn by-products, a mixture of animal, marine, and plant proteins (RUP mix), and animal meal increased the amount of non-ammonia, non-microbial N flow to the small intestine and enhance milk production compared with SBM control diets. Diets supplemented with corn by-products and fish meal had greater flow of Met to the small intestine more efficiently in comparison with other feed ingredient sources of RUP. Maiga et al (1996) reported that blood meal, meat and bone meal, corn gluten meal and expeller soybean meal contained high quantity

RUP compared with solvent extracted soybean meal and can potentially provide high quality amino acids (AA) to complement microbial AA for production. Bateman et al. (1999) reported the lactational performance will not be affected by the source of protein and rumen protected methionine (RPMet) and rumen protected lysine (RPLys), when fed at the levels of crude protein intake exceeding requirements relative to milk production. Hussein and Jordan (1991) reported that feeding fish meal as a protein supplement improved BW gain, milk yield and milk protein concentration. Aderibigbe and Church (1983) reported higher final BW weights, live and carcass ADG, and dressing percentages for animals fed hair and feather meals as protein supplements. In addition these researchers reported that the costs of gains were lower for cattle fed hair and feather meals as compared to those fed cottonseed meal.

TRUE PROTEIN

Although the ruminant has the capacity to utilize non-protein nitrogen (NPN) to meet some of its protein needs, Brito et al. (2007) reported that it is necessary to supplement true protein to optimize microbial protein and RUP supply to meet the metabolizable AA requirements of high producing dairy cows. Brito and Broderick (2007) reported that milk yield, milk composition, feed efficiency, and apparent N efficiency was greatly improved when supplements providing primarily true protein were fed compared with urea. Broderick et al. (1993) reported that cattle fed the true protein supplements had lower concentrations of ruminal ammonia and urea in milk and blood suggesting lower ruminal degradability. Results of these trials indicated that true protein supplements increased milk yield when low dry matter alfalfa silage was fed with corn silage as half of the TMR forage.

NON-PROTEIN NITROGEN

In general, NPN supplements such as urea and biuret can be used to meet a portion of the protein requirements, but they are less efficient than plant proteins in supporting animal growth and production (Shirley, 1986). When protein is deficient in the diet, NPN could be used for maintenance and growth. However, when protein is adequate, urea was utilized inefficiently (Harris and Mitchell, 1941). AAFCO (1955) suggested that not more than 1/3 of the total CP should be provided by NPN to prevent ammonia toxicity. Other NPN compounds that have been utilized by rumen microbes are ammonium, glutamine, glycine, and melamine. Johnson et al. (1973) reported that it takes time to adapt animals to NPN. The activity of rumen contents to hydrolyze biuret in vitro was at 42 days compared with 14 or 21 days. The ammonia appearance during the biuretolytic test was more marked on day 84 than days 42 and 66. Currier et al. (2004) reported that when dietary protein concentration is low, supplementing NPN supports satisfactory animal performance. The intake and digestibility of DM, OM, and N were greater for the lambs and cows when diets were supplemented with NPN compared with that of lambs and cows fed diets without any supplement N (CON). Plasma urea-N concentrations were increased with NPN supplementation compared with CON. These researchers also reported that total OM and N intake, duodenal flow of N, and bacterial efficiency were increased when diets fed to steers were supplemented with NPN. These results indicate that NPN supplementation can be effective for lambs, cows and steers when consuming low-quality forage.

ESSENTIAL AMINO ACIDS

Essential amino acids (EAA) are AA that are not synthesized or synthesized in adequate amount to meet requirements and must be supplied in the diet. Kajikawa (2002) reported

increased growth rate of ruminal bacteria in vitro when supplemental AA mixtures were provided. Scheia et al. (2005) reported increased energy-corrected milk (ECM) yield, milk protein yield and mobilization of fat for high producing dairy cows in early lactation with low energy intake, when feed increasing amounts of metabolizable amino acids absorbed in the small intestine. These results indicate that EAA supply in early lactation is important for up-regulating milk yield and milk protein yield. Aikman et al. (2002) reported that infusing EAA into abomasum or in the mesenteric vein increased milk protein concentration and yield, whereas milk lactose concentration decreased. Recovery of EAA as increased milk protein in milk was similar for both intravascular and abomasally infusion.

RUMEN DEGRADABLE PROTEIN

Rumen-degradable protein (RDP) is the protein that is degraded in the rumen and utilized by rumen microbes to make microbial protein. Approximately 40-60% of zein protein escapes rumen of sheep indicated that a substantial portion of its protein is degraded in the rumen (Mcdonald, 1954). The amount of RDP depends mainly on the solubility of protein in the rumen (Little et al. 1963). Santos et al. (1984) reported that in a diet supplemented with soybean meal, protein degradation in the rumen of lactating dairy cows was higher than for diets supplemented with corn gluten meal, wet brewers grains or distillers dried grains with soluble. Griswold et al. (2003) reported microbial growth efficiency was improved by urea infusion and increased RDP concentration. Cyriac et al. (2008) reported feeding 7.6% RDP lower milk yield as compared to 8.8%, 10.1% and 11.3%. Dietary RDP had no effect on body weight or percentages of milk fat, protein and lactose. Reducing the dietary RDP content to 7.6% had no effect on alter plasma concentrations of EAA, but resulted in decreases concentrations of milk urea-N. The apparent

efficiency of N utilization for milk N production was increased from 27.2% to 38.6% as RDP decrease from 11.3 to 7.6%. When RDP was 8.8% of DM, milk yield was not affected, which is in contrast from the predictions of the NRC (2001) model. Results of this trial indicated that, for mid-lactation dairy cows, current NRC recommendations for RDP may be overestimated.

RUMEN UNDEGRADABLE PROTEIN

In early lactation, dietary protein requirements are high because DMI is low. To satisfy the high requirement, it is not reasonable to feed more protein, which may result in wasteful excretion of excess nitrogen and increase environmental concerns. The more efficient way is to simultaneously maximize microbial synthesis of protein and minimize the breakdown of dietary protein in the rumen. Research on protein nutrition of ruminants has focused on improving the post-ruminal quantity and quality of AA. When the AA quality of a ration is low, efforts should be directed at promoting the utilization of rumen microbes to synthesize high-quality microbial protein. In contrast, when dietary AA quality of a ration is high, efforts should be directed toward supplementing increased protein that bypass the rumen (Shirley, 1986). The most economical RUP products that are resistant to rumen degradation but digested in the small intestine allowing for AA absorption are the most suitable. Without protection supplemental L-HCL-lysine is not effective for improving AA flow to intestine (Bernard et al., 2004). Ruminal NH_3 concentrations were typically not limiting for rumen microbial protein synthesis. Supplementing RUP in young ruminants' diets increases the protein and AA flow to the lower gastrointestinal tract (Coomerl et al., 1993; Harald, 1999). Increasing dietary RUP: RDP ratio from 5.2:11.6 to 7.1:9.5 improved nitrogen metabolism and milk production in early lactation

cows, but reduced blood urea linearly, suggesting potential reproductive benefits (Jahani-Moghadam et al., 2009).

Regarding environmental issues, improving nutrient utilization efficiency and performance of dairy cows through the use of RUP could reduce N release into the environment. Dinn et al. (1998) suggested that supplemental RPLys and RPMet could be used to lower N intake and improve N utilization reducing urine and fecal excretion of N into the environment. Canale et al. (1990) reported that supplementing rumen protected amino acids would potentially alleviate milk protein depression typically observed with fat supplemented diets. Nichols et al. (1998) observed that supplemental RPLys and RPMet in corn distillers' grains diets can increase milk and milk protein yield. For high producing dairy cows, supplementing RPMet and RPLys increased yields of milk protein and fat throughout the complete lactation (Robinson, 1995). Supplementing RPMet and RPLys can increase N absorption efficiency of growing lambs and improve performance of growing steers, while not influencing finishing steer performance (Oke et al., 1986). Feeding a basal diets supplemented with RPMet and RPLys to early lactation cows increases the yield of ECM, milk true protein and milk fat and decreased concentrations of plasma glucose (Armentano et al., 1993; Piepenbrink et al., 1996; Robinson et al., 1998; Socha et al., 2005) Supplementing RPLys and RPMet in diets supplementing with corn gluten meal and urea diet increased yield of milk and milk protein. However, when these RPLys and RPMet were included in diets supplemented soybean meal, no differences were observed in yield of milk and milk protein, although the milk protein percentage was increased. Plasma concentrations of methionine and lysine were improved when diets were supplemented with RPLys and RPMet (Rogers et al., 1989). Colin-Schoellen et al. (1995) reported that supplemental RPMet and RPLys did not affect DMI, milk yield, fat content, casein as a percentage of true protein or milk urea

concentrations, but increased milk protein yield and true protein percentage. Also these researchers found that glucose, urea, NEFA, BHBA and total free AA concentration in the plasma were not affected, but blood concentrations of Met and Lys increased slightly.

Broderick et al. (2008) reported that supplying RPMet in the lower CP diets, N efficiency is improved due to reducing urinary N excretion. However, when the cows were in negative N balance, because of reduced low DM intake, no response to supplemental RPMet was observed. With supplying RPMet, increased apparent N efficiency and protein yield was observed when diets based on ensiled alfalfa were supplemented with RPMet (Broderick and Muck, 2009). Supplementation of RPMet increased DMI, fat-corrected milk and fat yield, and tended to increase milk fat content, milk and milk protein yield (Broderick et al. 2009).

Supplementing RPLys increased the flow and percentage of lysine in duodenal digesta and plasma without effecting DMI (Blauwiekel, et al., 1997). These researchers also reported increased the percentage of milk protein with supplemental Lys. Bertrand et al. (1998) reported no differences were observed in DMI, yields of milk, milk fat, fat corrected milk, and energy-corrected milk with RPMet and RPlys supplementation. However, these researchers found that the percentage of milk protein, total N, and casein N were increased. Furthermore, they found that Plasma concentrations of methionine increased, but not lysine, indicating that lysine was the most limiting.

Two forms of rumen protected AA have been developed, the first is based on synthetic polymers and the second is based on a fatty acid lipid coating. In recent years, various sources of lipid coatings have been studied including combinations of unsaturated and saturated lipids. Hristov et al. (2005) did not observe any differences in ruminal fermentation, protozoa counts, and microbial protein flow to the duodenum and apparent nutrient digestibility when either

linoleic, a polyunsaturated, or oleic acid, a monounsaturated fatty acid, safflower rich oil was fed to beef cattle. Stearic acid (C18:0) is a saturated fatty acid without double bonds between adjacent carbon atoms, which means the hydrocarbon chain is flexible in shape and can be derived from the hydrogenation of the double bond of oleic acid. It is frequently found in hydrogenated vegetable or animal oils. Hydrogenation is a chemical reaction to reduce or saturate organic compounds by the addition of hydrogen. According to Mosley et al. (2002), biohydrogenation of oleic acid (C18:1) is not only directed to form stearic acid but involves formation of several positional isomers of trans monoenes.

Hydrogenated fatty acids, which have been commonly used in the coating, are poorly digested in the small intestine compared with unsaturated fatty acids (Pantoja et al., 1996; Harvatine and Allen, 2006). Previous research has shown that inclusion of unsaturated fatty acids (PFA) such as oleic acid along with a primarily saturated fatty acid may improve overall fatty acid digestibility in the small intestine (Drackley et al., 2007).

In situ and In vitro technique

In situ and in vitro are two ways to investigate the rumen degradability. In recent years, more in vitro trails have been conducted to compare degradability results as compared to in situ experiments. However, the in situ may be a more appropriate procedure to represent in vivo degredation. The Dairy NRC 2001 recommends using the in Situ procedure to measure feed RUP values. Degradation rates determined with exogenous enzymes including S. griseus, ficin, and neutral proteases with amylase methods are not as consistently related to those concluded by the in situ technique (Roe et al., 1991).

The objective of this study is to employ situ and vivo procedures to investigate the capability of oleic acid and stearic acid to ruminally protected lysine and provide its intestinal absorption. We hypothesized that oleic acid would have higher rumen escape and intestinal absorption.

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CHAPTER 3

RUMINAL ESCAPE AND INTESTINAL DIGESTIBILITY OF EXPERIMENTAL RUMINAL PROTECTED LYSINE SUPPLEMENTS

ABSTRACT

Three trials were conducted to determine the ruminal escape and intestinal absorption of experimental ruminal protected lysine supplements using the mobile bag technique. In each trial, lactating Holstein cows previously fitted with ruminal and duodenal cannula were used in the mobile bag studies as outlined by NRC (2001). For each trial, 5 g of supplement treatment was weighed into each of twenty 10 x 20 cm nylon bags and incubated for 16 h. Upon removal from the rumen, bags were washed, dried and weighed. Residues were composited by treatment within cow. Approximately 0.8 g of residue for each treatment was repackaged into 5 x 10 cm nylon bags (20 per treatment), soaked in pepsin/HCl solution for 2 h before inserting into the duodenum and subsequently collected in the feces. Samples were analyzed for DM, N, LYS and acid hydrolysis fat concentrations. In trial one, five experimental treatments were prepared using two sources of hydrogenated oil (soybean oil or rapeseed oil) with 0 or 2% oleic acid in the hydrogenated oil coatings. No differences were observed in the ruminal escape of DM and N. Inclusion of oleic acid in the coating improved intestinal digestibility of DM and N. The observed decrease ($P<0.0001$) in intestinal digestibility of DM and N was especially marked with the rapeseed oil that did not contain any oleic acid. In trial two, the two experimentally products were compared with three commercially available ruminally protected lysine products. The N and lysine in the experimental products were more ruminally protected, but less intestinally digestible ($P<0.10$). The N and lysine in the commercial available products were less ruminally protected, but highly intestinally digestible ($P<0.10$). In trial three, four experimental products varying in the proportion of lysine (55 and 58%) and oleic acid (2 or 4%) were compared in a 2 x 2 factorial arrangement to determine the effect of increasing lysine content and addition of oleic acid in the coating. The percentage of N and lysine escaping the rumen decreased increased as

oleic acid increased from 2 to 4% (P<0.05) and as the proportion of supplemental lysine increased from 55% to 58% (P<0.05). No differences were observed in intestinal digestibility of N or lysine in response of increasing lysine content or addition of oleic acid (P<0.05). These trials indicate that the proportion of lysine in the product and composition of the fat coating influences ruminal escape and intestinal digestibility of N and lysine.
KEY WORDS: rumen protected lysine, ruminal escape, intestinal digestibility

INTRODUCTION

Controlling the type and quantity of dietary protein is important for optimizing milk yield and minimizing N as waste into the environment (Dinn et al., 1998). In ruminants, the micro-organisms in the reticulo-rumen degrade a substantial portion of dietary protein to ammonia and peptides. Depending on availability of readily fermentable carbohydrates, a substantial portion of ruminally degradable N is used to synthesize microbial protein. The excess rumen ammonia N is absorbed from the rumen, metabolized to urea in the liver, and either recycled back to the rumen through saliva across the rumen wall or excreted as urinary N. Microbial protein passes out of the rumen into the small intestine where it is 80% digested and its peptides and amino acids absorbed actively in the jejunum and ileum. Microbial protein is very high quality because of desirable ratio of Lysine: Methionine. The proportion of rumen undegradable protein that enters the small intestine is largely digested and absorbed and provides additional supplemental essential amino acids that are useful in meeting the N requirements of higher producing (>30kg/d) lactating dairy cows. The quantity and quality of the dietary protein escaping ruminal degradation is an important consideration for supplementing higher producing lactating dairy cows with essential amino acids to meet their overall requirements.

Lysine and methionine are considered co-limiting amino acids for growth and milk synthesis in dairy cattle (Clark, 1975; Schwab et al., 1976; Nichols et al., 1998; Socha et al., 2005). Supplementing crystalline forms of essential amino acids is not practical for ruminants as compared to non-ruminnats because of their rapid and extensive degradation by rumen micro-organisms. This has encouraged a considerable amount of research over the past three decades to develop technologies to protect amino acids from ruminal degradation. Ruminal protected methionine has been available for feeding ruminants for several years (Papas et al., 1984; Overton et al., 1998; Patton, 2010.) which allows more precise formulation, but technology for protecting lysine and other amino acid have not been available until recently.

In recent years, various groups have developed lipid coatings to protect supplemental lysine from rumen microbial degradation (Ardaillon et al., 1989; Ardaillon and Franzoni, 1992; Rappoport, 1999). Althoug, these coatings provide protection from rumen microbial degradation (Smith and Boling, 1984; Wu and Papas, 1998), however, less is known regarding the digestion and the availability of EAA in the small intestine with such coatings. Hydrogenated fatty acids, which have been used as rumen degradation protective coating, are poorly digested in the small intestine as compared with unsaturated fatty acids (Pantoja et al., 1996; Harvatine and Allen, 2006). Previous research has shown that including of polyunsaturated fatty acids (PFA) such as oleic acid along with a primarily saturated fatty acid will improve its overall digestibility of fatty acid in the small intestine (Drackley et al., 2007).

In vitro, in situ, and enzymatic techniques have been used to evaluate ruminal degradability and intestinal digestibility. In situ techniques are considered the standard for measuring RUP of feedstuffs (NRC, 2001), but this approach is laborious, time intensive and expensive. RDP coefficient rates as determined by methods, using neutral protease enzymes

isolated from such as, from S. griseus and ficin with amylase are not consistently related to those concluded by the situ technique (Roe et al., 1991).

The objective of this research is to determine the effect of adding various percentages of oleic acid to a hydrogenated fat coating applied to experimental supplements containing different proportions of supplemental lysine on ruminal escape and intestinal digestibility of lysine employing the mobile bag technique and to evaluate the ruminal escape and intestinal lysine digestibility of several commercially available products.

MATERIALS AND METHODS

Animals

All methods were review and approved by the University of Georgia Animal Care and Use Committee prior to conducting the trial. Two Holstein cows previously fitted with flexible ruminal and duodenal cannulas were used for the mobile bag trials as according to NRC (2001). The cows were housed in an individual pen and fed a total mixed ration once daily at 0700 h formulated to meet NRC (2001) recommendations. The diet was composed of (% of total DM): 33.11% corn silage, 9.35% ryegrass silage, 4.68% alfalfa hay, 6.75% whole cottonseed, 12.63% wet brewers grains, 17.77% ground corn, and 15.71% concentration containing supplemental protein, minerals, and vitamins. The cows have free access to water at all times.

Mobile Bag Technique

Ruminal degradability was measured in situ with product subjected to 16 h of rumen incubation. Approximately 5 g of each test product was weighed into 10 x 20 cm nitrogen-free nylon bags with a pore size of 50±15 microns (Ankon Technology, Macedon, NY) . The top of

each bag was heat sealed three times to prevent the sample from leaking. Soybean meal (48% CP, solvent extracted) was included as a stand to validate ruminal degradability measurement. Twenty bags are prepared for each test product per cow. At 1600 h, a laundry bag with 20 bags of each test material plus five blank bags per cow were inserted through the ruminal cannula into the ventral sac of the rumen and incubated for 16 h (Kononoff et al., 2007). After ruminal incubation, all bags were removed and immediately hand rinsed in cold water to stop microbial activity then machine washed (Roper Home Appliances Model RTW4100WQ0, Whirlpool Corporation, Chicago, IL) to remove any microbial or feed contamination. Following washing, all bags were dried in a forced air oven (45°C) and weighed. After weighing, samples are composited by each test product for each cow.

Intestinal digestibility was determined by weighing 0.8 g of the composited residue remaining after ruminal incubation into 5 x 10 cm nitrogen-free nylon bags with a pore size of 50±15 microns (Ankon Technology, Macedon, NY). Eighteen bags were prepared for each treatment and bags were sealed as described. Bags were initially incubated in a pepsin - HCl solution (100 mg pepsin/L of 0.01 N HCl) for 2 hours at 39°C in a shaking water bath to simulate abomasal digestion. HCL was added to decrease the pH to 2.4. After incubation, bags were rinsed with distilled water and kept at -18°C until bags were introduced into the duodenum of cannulated cows. Bags were inserted into the duodenum through the duodenal cannula beginning at 0900 h. One bag was inserted every 15 minutes to permit movement of previous bags into the intestine and avoid compaction. Each session lasted 3 h followed by a minimum of 8 h rest before the next session. This process was repeated until all bags had been inserted into the duodenum. Bags were recovered from the feces approximately 8-16 h after initial insertion. Collected bags were thoroughly rinsed by hand in cold water to remove any fecal material and

dried in a forced air oven (45°C). After drying, bags were weighed to determine residue weight. Samples were composited by treatment within cow for subsequent nutrient analysis.

Samples of raw material, ruminal and fecal composited samples for each test product were analyzed for DM, N, lysine, and acid hydrolysis fat (AOAC, 2006) concentrations. Rumen escape percent was calculated as residue remaining after rumen incubation divided by the amount of raw material weighed into the bag. Intestinal digestibility was calculated as 1 minus the residue remaining in the bag after intestinal digestion and fecal excretion divided by the amount of ruminal residue weighed into the bag.

Trial 1

A total of five experimental treatments differing in source of hydrogenated oil used to coat the product, concentration of oleic acid in the coating and lysine concentration were tested. Three experimental products were produced using hydrogenated rapeseed oil (G4, G6, and G8) as the primary component in the coating and two experimental products were produced using hydrogenated soybean oil (A4 and A6). The composition of the experimental products is outlined in Table 1. Experimental products A4 and A6 produced by different runs were prepared using hydrogenated soybean oil plus oleic acid and represent different batches of the same formulation. Experimental products G4, G6 and G8 were prepared using hydrogenated rapeseed oil with 0 or 2% oleic acid applied to either 55% or 60% of lysine sulfate.

Ruminal escape and intestinal digestibility DM, and N data were subject to analysis of variance using SAS (version 9.1). The model included effects of cow and treatment. Contrast statements were included to evaluate the effects of the source of oil (soybean or rapeseed), and

proportion of oleic acid in the rapeseed coatings (0 or 2%) in the G4, G6, and G8 products. Significance was declared when $P < 0.10$.

Table 1. Ingredient and chemical composition (% of DM) of experimental ruminally protected lysine supplements differing in source of hydrogenated oil and proportion of oleic acid included in the coating.

	A4	A6	G4	G6	G8
Ingredient composition, %					
Lysine sulfate	60	60	60	55	60
Hydrogenated soybean oil	36	36			
Hydrogenated rapeseed oil			36	43	36
Oleic acid	4	4	0	2	2
Steric acid	0	0	4	0	2
Chemical composition, % of DM					
N	6.68	6.78	7.36	6.94	7.83

Trial 2

The ruminal escape and intestinal digestibility of DM, N, lysine, and fat of two experimental products (A and B) and five commercially available supplements (C, D, and E) were evaluated. The composition of the products is outlined in Table 2. Data were subjected to analysis using PROC GLM procedure of SAS (Version 9.1). The model included effects of cow and treatment. When treatment differences were detected ($P < 0.10$), the PDIFF option was used for mean separation.

Table 2. Chemical composition (% of DM) of experimental and commercially available protected lysine supplements.

	A	B	C	D	E
N	6.80	7.34	3.10	6.53	8.13
Lysine	29.46	32.44	16.18	33.07	42.84
Fat[1]	40.16	22.6	65.54	36.32	31.43

[1]Acid hydrolysis fat.

Trial 3

In Trial 3, four experimental products were produced for the trial with a 2 x 2 factorial arrangement of treatments to provide two concentrations of lysine (55 [L55] or 58% [L58]) and two concentrations of oleic acid (2 [O2] or 4% [O4]) in the final product. The composition of the products is outlined in Table 3. Lysine sulfate was coated with a blend of hydrogenated rapeseed oil plus oleic acid by IPC Process Center GMBH & Co., KG (Dresden, Germany) using a proprietary process. The product was shipped to the University of Georgia - Tifton Campus for the trial. Data were subjected to analysis of variance using PROC GLM procedures of SAS (Version 9.1). The model included cow, the main effects of lysine and oleic acid content and their interaction, and error. When an interaction was detected, the PDIFF option was used for mean separation. Significance was declared when $P < 0.05$.

Table 3. Ingredient and chemical composition of experimental protected lysine supplements differing in lysine content and oleic acid.

	L55O2	L55O4	L58O2	L5804
Ingredient Composition, %				
Lysine sulfate	55	55	58	58
Hydrogenated fat[1]	43	41	40	38
Oleic acid	2	4	2	4
Chemical composition, % of DM				
N	6.85	6.86	7.24	7.17
Lysine[4]	29.09	29.41	29.65	30.08
Fat[2]	43.99	43.70	41.57	41.67
C16:0[3]	2.71	2.58	2.52	2.39
C18:0[3]	38.96	37.19	36.24	34.47
C18:1[3]	1.56	3.12	1..56	3.12
C22:0[3]	0.22	0.21	0.20	0.19

[1]Average composition: 97.7% fat with 0.10% C14:0; 6.30% C16:0; 90.50% C18:0; 2.00% C20:0; 0.50% C22:0; and 0.30% C24:0.

[2]Acid hydrolysis fat.

[3]Calculated based on composition of hydrogenated fat and oleic acid and expresses as a proportion of total fatty acids.

[4]Average of lysine % of L5502 and L5504 is 29.25. Average of lysine % of L5802 and L5804 is 29.865, which is approximately 2.1% higher than 29.25.

RESULTS

Trial 1

The ruminal escape and intestinal digestibility of experimental lysine supplements coated with either hydrogenated soybean or rapeseed oil and with or without oleic acid are presented in Table 4. Ruminal escape of DM was similar among five treatments ranged from 84.40% to 92.20%. Treatment G4 had lowest intestinal digestibility of DM and N ($P<0.0001$). This

indicates that the oleic acid supported improved intestinal digestibility. G6 and G8 had highest (P<0.0001) intestinal digestibility of N, which were 98.9% and 98.9% compared with A4 and A6 with 90.9% and 97.3%. This indicated coated with hydrogenated rapeseed oil and oleic acid had better intestinal digestibility. Ruminal escapes of DM were 92.2% and 88.6% for G6 and G8 respectively, which were numerically higher than A4, A6 and G4 with 88.4%, 92.0% and 84.4% respectively. Ruminal escapes of N were 100.0% and 80.1% for G6 and G8 respectively, which were also numerically higher than A4, A6 and G4with 86.3%, 87.2% and 81.9% respectively. These indicated hydrogenated rapeseed oil with oleic acid coating had better protection from rumen degradation. Treatment G6 had numerically highest ruminal escape of DM and N, which are 92.2% and 100.0% respectively, and may result from a greater proportion of fat coating and a lower concentration of lysine.

Table 4. Ruminal escape and intestinal digestibility of experimental lysine supplements coated with either hydrogenated soybean or rapeseed oil with or without oleic acid. (Trial 1)

	Treatments						Contrast[2]	
	A4[1]	A6	G4	G6	G8	SE	Fat Source	Oleic Acid
Ruminal escape, %								
DM	88.4	92.0	84.4	92.2	88.6			
N	86.3	87.2	81.9	100.0	80.1			
Intestinal digestibility, %								
DM	56.6	60.6	47.8	65.5	60.4	0.81	0.36	<0.0001
N	90.9	97.3	74.5	98.9	98.9	0.25	<0.0001	<0.0001

[1]A4 = 60% lysine sulfate, 36% hydrogenated soybean oil and 4% oleic acid.

A6 = 60% lysine sulfate, 36% hydrogenated soybean oil and 4% oleic acid.

G4 = 60% lysine sulfate, 36% hydrogenated rapeseed oil and 0% oleic acid.

G6 = 55% lysine sulfate, 43% hydrogenated rapeseed oil and 2% oleic acid.

G8 = 60% lysine sulfate, 36% hydrogenated rapeseed oil and 2% oleic acid.

[2]Contract statements: Fat Source = hydrogenated soybean oil versus hydrogenated rapeseed oil; and Oleic acid = 0 versus 2% in the rapeseed oil coating.

Trial 2

The ruminal escape and intestinal digestibility of protected lysine supplements are presented in table 5. The proportion of N and Lysine escaping rumen were higher (P <0.1) experimental protected lysine A and B compared with commercial available products C, D and E. The intestinal digestibility of N and lysine of experimental products were lower than the commercial available products (P<0.10). The calculated metabolizable lysine content of the products based on their ruminal escape and intestinal digestibility was: 62.0, 48.5, 31.9, 37.7, and 60.7% for A, B, C, D, and E, respectively.

Table 5. Ruminal escape[1] and intestinal digestibility of protected lysine supplements. (Trial 2)

	A[2]	B	C	D	E	SE	P
Ruminal Escape, %							
DM	97.8[a]	86.0[ab]	83.4[b]	61.5[c]	79.9[b]	4.4	0.0275
N	92.8[a]	82.7[a]	36.5[b]	35.0[b]	52.5[b]	8.8	0.0278
Lysine	93.8[d]	81.1[d]	37.8[e]	37.7[e]	61.0[e]	12.5	0.1022
Fat	79.8[bc]	100.0[a]	100.0[a]	73.7[c]	93.4[ab]	4.6	0.0436
Intestinal Digestibility, %							
DM	37.4	34.6	29.1	38.1	43.8	2.6	0.0955
N	62.8[c]	57.1[c]	81.1[b]	97.9[a]	99.0[a]	2.4	0.0007
Lysine	66.1[c]	59.8[c]	84.3[b]	99.9[a]	99.5[a]	3.6	0.0040
Fat	43.1	36.2	20.5	37.1	31.1	6.9	0.3490

[1]Measured after 16 h incubation in the rumen.

[abc]Means with unlike superscripts in the same row differ ($P < 0.05$).

[de]Means with unlike superscripts in the same row tend to differ ($P < 0.10$).

[2]A and B are experimental products. C, D and E are commercial available products.

and lysine ($P = 0.01$) because proportion escaping the rumen was higher for L55O2 and L55O4 than L58O4.

Trail 3

Ruminal escape and intestinal digestibility of experimental lysine supplements are presented in Table 6. The proportion of DM escaping rumen decreases as both the proportion of supplemental lysine in the product increased ($P = 0.001$) from 55% to 58% and as the percentage of oleic acid composition increased ($P = 0.005$) from 2% to 4%. There was an interaction between the proportion of lysine and oleic acid for ruminal escape of N ($P = 0.05$).

No differences were observed in the intestinal digestibility of DM, N, lysine or fat among treatments (Table 6). The calculated flow of lysine to the small intestine when 100 g of raw product was introduced into the rumen was lower for F58O4 compared with the other treatments resulting in an interaction between the proportion of lysine and oleic acid in the amount of lysine flowing to small intestine ($P = 0.01$). Total grams of lysine that were digested in the small intestine did not differ among treatments.

Table 6. Ruminal escape[1] and intestinal digestibility of experimental protected lysine supplements containing 55 or 58% lysine sulfate with 2 or 4% oleic acid in the coating. (Trial 3)

	Treatment					P		
	L55O2	L5504	L58O2	L58O4	SE	Oleic	Lysine	Int
Ruminal Escape, %								
DM	94.2	92.0	90.0	86.5	0.4	0.005	0.001	0.16
N	89.9[a]	89.3[a]	85.0[ab]	75.6[c]	1.4	0.04	0.007	0.05
Lysine	86.2[a]	86.2[a]	89.2[a]	74.2[b]	1.3	0.01	0.04	0.01
Fat	94.9	92.6	84.3	77.8	1.9	0.10	0.006	0.34
Intestinal Digestibility, %								
DM	23.3	27.0	25.7	29.8	2.3	0.18	0.33	0.92
N	38.3	38.7	41.2	47.9	3.3	0.36	0.17	0.42
Lysine	44.5	47.8	43.7	53.3	5.9	0.46	0.56	0.48
Fat	28.1	29.2	27.0	24.3	4.2	0.86	0.52	0.68
Lysine								
Flow to SI, g	25.1[a]	25.3[a]	26.4[a]	22.3[b]	0.4	0.02	0.13	0.01
Digested in SI, g	11.2	11.4	11.5	11.9	1.4	0.85	0.77	0.96

[1]Measured after 16 h incubation in the rumen.

[2]L55O2 = 55% lysine sulfate and 2% oleic acid; L55O4 = 55% lysine sulfate and 4% oleic acid; L58O2 = 58% lysine sulfate and 2% oleic acid; and L58O4 = 58% lysine sulfate and 4% oleic acid.

[abcd]Means with unlike superscripts in the same row differ ($P < 0.05$).

DISCUSSION

Limited data are available on the characteristics of ruminally protected lysine supplements. The ruminal escape of lysine in the products evaluated in these trial were higher than the values reported by Watanabe et al. (2003) who observed a recovery rate of lysine in the

abomasal outflow of 58.3 ± 4.1%. Swanepoel (2009) reported ruminal lysine escape of 19 to 24%.

Addition of 4% oleic acid increased ruminal degradability of N and lysine compared with products with 2% oleic acid suggesting that the hydrogenated fat coating was more susceptible to ruminal degradation as oleic acid increases. This is consistent with observations Harvatine and Allen (2006) who reported linear increases in ruminal fatty acid digestibility with increasing unsaturated fatty acid supplements. We did not observe any difference in intestinal digestibility of fat as oleic acid percentage increased in trial 3. Intestinal digestibility of N and lysine were numerically highest for the product with 58% lysine and 4% oleic acid suggesting that the higher oleic acid content could potentially support improved digestibility. Drackley et al. (2007) reported that the total fatty acid digestibility increased linearly as infused oleic acid increased.

Watanabe et al. (2003) reported that average ruminal escape of 58.3% and intestinal digestibility of 49.5% for experimental ruminally-protected lysine supplements providing 28.9% metabolizable lysine. Swanepoel (2009) reported that intestinal digestibility of 96% and calculated a metabolizable lysine content of only18.2 to 23.0%. In Trial 3, metabolizable lysine concentrations were 38.4, 41.2, 39.0, and 39.5% for L55O2, L55O4, L58O2, and L58O4, respectively. Although ruminal escape was lower for L58O4, intestinal digestibility was numerically higher resulting in similar metabolizable lysine concentrations. The metabolizable lysine content of the experimental and commercial products tested in Trial 2 ranged from 31.9 to 62.0%.

The experimental supplements in our study have a higher rumen bypass rate than reported for commercial products currently marketed in the US. The intestinal digestibility of lysine was much higher for the commercial products than the experimental products (Trial 2).

Much of the difference in metabolizable lysine concentrations of the commercial products is related to differences in ruminal escape rather than any difference in intestinal digestibility. The composition of the coating used to protect these products is not reported, so it is not possible to compare these directly with the experimental products examined in our trials.

CONCLUSIONS

Results of these trials suggest that hydrogenated fat can be used to coat lysine and provide protection from ruminal degradation, but the coating is also resistant to digestion in the small intestine which reduces amount of lysine actually available for absorption. Inclusion of oleic acid in the coating does appear to improve intestinal digestibility increasing potential metabolizable lysine concentrations. However, increasing both the concentration of lysine and oleic acid increase susceptibility to ruminal degradation and reduce the amount of lysine that is delivered to the small intestine. The three commercial products which were tested vary in their metabolizable lysine content. Data on the metabolizable lysine content of protected lysine supplements is important for formulating rations that can be fed to lactating dairy cows to improve N efficiency and production.

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CHAPTER 4

CONCLUSIONS

Results of these trials suggest that hydrogenated fat can be used to coat lysine and provide protection from ruminal degradation, but the coating is also resistant to digestion in the small intestine which reduces amount of lysine actually available for absorption. Inclusion of oleic acid in the coating does appear to improve intestinal digestibility increasing potential metabolizable lysine concentrations. However, increasing both the concentration of lysine and oleic acid increase susceptibility to ruminal degradation and reduce the amount of lysine that is delivered to the small intestine. The three commercial products which were tested vary in their metabolizable lysine content. Data on the metabolizable lysine content of protected lysine supplements is important for formulating rations that can be fed to lactating dairy cows to improve N efficiency and production. Additional research is needed to evaluate the effects of coatings that effectively protect lysine from ruminal degradation but allow higher digestion in the small intestine to increase metabolizable lysine content.