Effects of ruminally-protected lysine supplementation on growing and finishing performance of beef cattle

by

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Abstract

Three experiments were conducted to evaluate impacts of supplementing ruminally-protected lysine (RPL) to growing and finishing cattle. In experiment 1, 448 heifers (287 ± 14.1 kg body weight (BW)) were used to evaluate backgrounding performance of cattle fed RPL (SafeGain™, H.J. Baker & Bro. LLC., Shelton, CT). Treatments were RPL supplemented at 0, 15, 30, or 45 g/animal daily. Heifers were blocked by BW and randomly allocated to 16 blocks of 4 pens each for a total of 64 pens containing 7 heifers/pen. At the end of the 112-day backgrounding period, a subset of 12 blocks were consolidated, such that 2 pens from each backgrounding treatment were combined into one finishing pen. Cattle were weighed, relocated to finishing pens, and fed a common finishing diet (no supplemental lysine) for 95 days until harvest to evaluate carryover effects of RPL fed during backgrounding. In experiment 2, 384 steers (413 ± 29.2 kg BW) were used to evaluate effects of supplementing RPL (SafeGain, H.J. Baker & Bro.) in conjunction with a β-adrenergic agonist (BAA) on performance and carcass characteristics. Treatments were (2 x 4 factorial) 0, 20, 40, or 60 g/animal daily of RPL in conjunction with BAA during the last 42 days on feed (112 days total finishing period); and two step-up regimens: conventional 21-days without or an accelerated 10-days step-up with an oral dose of *Megasphaera elsdenii* probiotic (ME; Lactipro®, MS Biotec, Wamego, KS). Steers were blocked by BW and randomly allocated to one of 64 pens with 6 animals/pen. In experiment 3, 448 steers (352 ± 25 kg BW) were used to evaluate impact of ME, alone or in combination with RPL (USA Lysine, Kemin Industries Inc., Des Moines, IA), on performance and carcass characteristics. Steers were blocked by BW and randomly allocated to one of 64 pens (7 steers/pen). Treatments were arranged as a 2 x 2 factorial in a randomized complete block experiment, with treatments consisting of: RPL fed at 0 or 45 g/animal daily; and two step-up regimens as described for Exp.

2. Finishing diets were fed once daily for 144 or 172 days, *ad libitum*. At the end of all trials, cattle were weighed and harvested at a commercial abattoir, where carcass data were collected. In all studies, pen was the experimental unit, and block was the random effect. Backgrounding performance improved linearly in response to increasing amounts of RPL ($P \leqq 0.05$) in Exp. 1, and improvements realized during background were retained throughout finishing. In Exp. 2, there were no effects of RPL or ME on daily gain (ADG), dry matter intake (DMI), or gain:feed ($P > 0.45$); but liver abscess incidence was increased with RPL supplementation ($P < 0.05$; 28.3, 39.0, 46.9 and 39.4% for cattle fed 0, 20, 40 and 60 g/day of RPL, respectively). An interaction between RPL and ME was observed for hot carcass weight ($P = 0.01$). Dosing cattle with ME with an accelerated transition period decreased marbling score ($P = 0.03$) and yielded a lower percentage of carcasses grading Choice ($P = 0.03$) than those traditionally adapted. No other effects of ME or RPL were observed for carcass characteristics ($P > 0.10$). In Exp. 3, no interactions between ME and RPL ($P > 0.1$) were observed. Steers given ME consumed less roughage compared to their counterparts without ($P < 0.05$), but ADG, DMI, and gain:feed were similar ($P > 0.10$) among treatments. Administering ME tended to increase percentage of USDA Prime carcasses compared to control (2.7 vs 0.5% respectively; $P = 0.06$). Feeding RPL did not affect feedlot performance, hot carcass weight, or other carcass traits ($P > 0.10$) but tended to increase USDA Yield Grade ($P < 0.07$). Thus, supplementing RPL improved backgrounding performance, but no benefits were observed during feedlot finishing. Additionally, an accelerated step-up program with ME yielded finishing performance comparable to that of traditionally-adapted cattle.

Keywords: feedlot, *Megasphaera elsdenii*, ruminally-protected lysine

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Dedication

For my parents who taught me that the most valuable possession you have is your knowledge; it can't burn down, it can't break or be stolen. It will be always yours and with you, wherever you go.

Aos meu pais que me ensinaram que a maior riqueza da vida é o conhecimento. Conhecimento não pega fogo, não se quebra, nem pode ser roubado. O conhecimento adquirido será sempre seu e estará sempre contigo.

Chapter 1 - Review of Literature

INTRODUCTION

Amino acids (AA) are the single component (monomers) of protein molecules. Proteins are the most abundant and diverse of the cellular macromolecules (Lodish et al., 2000), where cells can contain several dozen different proteins with a variety of specialized functions. The word protein derives from the Greek word *proteios* which translates "the first rank", as a meaning of its importance in living organisms. Proteins are structural components of cells and tissues; they transport and store small molecules throughout the organism (e.g. oxygen transport by hemoglobulin), allow signaling between cell (e.g. hormones), defend the organism against antigen invasion (e.g. antibodies), and catalyze nearly all biological reaction in the form of enzymes. Amino acids can be classified in four categories according to their chemical properties and role in the protein structure: hydrophobic, polar, amphipathic, and charged. Amino acids also are categorized from the perspective of nutritional requirements as essential and non-essential. All animals require AA for optimum growth, reproduction, lactation and maintenance of general physiological functions (Kung and Rode, 1996). Ruminants can be considered self-sufficient for acquiring essential AA due to the production of microbial protein in the rumen, but AA supply may be insufficient under certain conditions in which productivity is relatively high. It is unlikely that microbial protein alone would be sufficient to meet all AA requirements of production animals expected to grow at their maximum capacity. In these cases, ruminants may need exogenous sources of AA to allow manifestation of their maximum potential for performance. The availability of dietary AA and efficiency of utilization determines the rate of protein accretion in growing animals (NRC, 1996). Understanding AA requirements, intestinal

supply, absorption in the small intestine (SI), and efficiency of utilization in ruminants are fundamental concepts to nutritionists that seek to optimize productivity of ruminants.

GROWTH DYNAMICS IN CATTLE

Various factors can influence body composition and nutrient utilization of growing cattle. Previous authors have identified physiological age, body weight (BW), sex, frame size, daily gain, breed type, hormonal status, nutritional management, and other dietary factors as considerations. Requirements for maintenance and growth are calculated separately and are considered to be affected differently by each of these factors. This occurs because maintenance requirements are calculated as a function of total BW (Fox and Black, 1984) or whole body protein weight (Johnson et al., 2012), while growth requirements are calculated based on fractions of protein, water, and fat tissue gain (Fox and Black, 1984; Johnson et al., 2012). As an animal ages, the composition of body fractions and tissue gain change according to energy availability (Johnson et al., 2012). Consequently, requirements for maintenance and growth are altered as animals mature. These tissue fractions are considered substantially different in terms of energy cost per 1 kg of BW gain. Protein growth is associated with accumulation of bodily water, as approximately 78% of muscle mass is composed of water. Water content of adipose tissue is not greater than 10% (Owens et al., 1995). As a consequence, fat deposition requires more energy than fat-free lean tissue (protein) deposition. Therefore, composition of tissue is an important variable when estimating energy requirements for growth. During animal maturation, whole-body fat fractions increase, and explains why energy demanded per unit of total BW gain is greater in older animals (Wright and Loerch, 1988). Thus, when comparing animals' requirements, it is suggested to consider animals with similar body composition, where the

proportions of fat tissue dictate similar energy requirements for tissue gain (Garrett et al., 1959). Capacity for fat storage in the body is dependent on bodily protein weight (Johnson et al., 2012). For this reason, proportion of body fractions dictates the composition of tissue gain and the corresponding energetic requirements for that gain.

When estimating energy cost to maintain each body fraction, other metabolic differences must be considered. Bodily protein mass is subject to turnover, which means constant catabolism occurs in muscle tissue; thus, in order to maintain protein reserves, the body needs to spend energy to resynthesize catabolized protein. Johnson et al. (2012) illustrated how energy requirements for protein growth peaks during the early stages of an animal's life. As bodily protein fractions increase, requirements for growth decline while requirements for protein maintenance increase. Contrastingly, fat tissue is regarded as energy storage for the body, and maintenance of fat tissue is considered to have a low energy requirement. Fat deposition occurs as a function of bodily protein weight (Johnson et al., 2012); hence it occurs post protein growth; meanwhile fat catabolism occurs when available energy cannot support maintenance requirements. Fat reserves used to supplement energy are mainly mobilized with the purpose of maintaining body tissue (i.e. degraded protein) and metabolic functions; however, fat catabolism was described to occur in ruminants near energy maintenance to sustain protein deposition (Ledger and Sayers, 1977). Lastly, when energy levels from intake and fat catabolism are insufficient to fulfill maintenance requirements, protein from the animal's bodily mass is utilized.

PROTEIN SUPPLEMENTATION AND AA UTILIZATION IN RUMINANT ANIMALS

Protein requirements and efficiency of AA utilization will vary according to each AA (essential vs nonessential), physiological use of protein source (i.e. maintenance or growth), available energy to support protein metabolism, AA profile of the diet, and the assay used to determine requirement and efficiency of AA utilization.

Protein requirements for maintenance and growth are predicted independently. Efficiency of protein utilization for maintenance is assumed to be greater than that for growth (Johnson et al., 2012). Protein requirements for maintenance are estimated based on losses of protein associated with production of endogenous feces, urine, and scurf, where efficiency of AA utilization is assumed to be equal across all essential AA. Branched-chain AA are an exception, and are considered to have lower use efficiency (Titgemeyer, 2003). Maintenance requirements for each specific AA are estimated based on AA profile of tissues, scurf losses are predicted based on keratin AA profile, while whole-body AA profile is used to estimate urinary and fecal losses. Titgemeyer (2003) pointed out that estimating AA catabolic losses via whole-body AA profile likely doesn't result in an accurate estimation.

Amino acid requirements for growth are assessed based on the protein profile of whole-body tissue. Efficiency of utilization for accrued bodily protein is considered different for each AA and can vary according to AA profile of the diet, as well as BW and age of the animal. Dietary AA can be classified relative to their availability in the diet, where AA are ranked in terms of amount required in the diet to promote protein growth. The rain barrel analogy is commonly used to describe the limiting AA concept. It describes protein synthesis dependence on the availability of limiting AA that restrict potential for growth when insufficient. Specific proportions of AA required within metabolizable protein (MP) fractions are predicted based on AA profile of whole-body tissue. Ainslie et al. (1993) developed an equation to predict the

efficiency of protein utilization for growth, where overall efficiencies decrease as an animal's weight increases. The equation does not, however, consider efficiency of AA utilization depending on overall AA availability.

Efficiency of AA utilization has been described as a relationship for which energy intake and AA deposition are linearly correlated (Campbell et al., 1985; Chowdhury et al., 1997; Titgemeyer et al., 2003). Amino acid requirements commonly are distinguished in two phases: (1) a protein-dependent phase where protein is limiting, thus increases in protein supply lead to increases in protein deposition, and (2) an energy-dependent phase, where energy supply is limiting and increases in energy intake result in increases in protein deposition. During the protein-dependent phase, increases in energy intake do not affect efficiency of AA utilization, but rather increase AA requirements for growth.

Correlation between energy intake and AA deposition can be challenging to quantify in ruminants. Ruminal microorganisms supply at least 50% of the total AA required by ruminants (Spicer et al., 1986; NASEM, 2016). When energy content in the diet is increased, ruminal microbial density also increases; consequently, protein supply to the animal is greater. Because of this relationship, it is difficult to separate energy supply, protein supply, and improvements in performance. Increases in dietary energy can lead to increases in performance via greater protein supply in the form of microbial cell protein, or by increasing efficiency of AA utilization by the animal.

Oldham and Alderman (1982) first estimated efficiencies of protein utilization for growth as no greater than 75%. Later, Ainslie et al. (1993) suggested that efficiency of protein utilization in growing animals (considering 40-kg calves) was no greater than 80%, and that it decreased with increases in animal BW. Titgemeyer (2003), Schroeder et al. (2007), and

NASEM (2016) described protein utilization in growing cattle as bell-shaped, where protein deposition linearly increases with an increase in protein supply during the protein-dependent phase, plateaus as energy becomes limiting, and decrease as protein supply becomes excessive, with a decline in efficiency being no less than 30%. Although researchers have attempted to estimate an average value for efficiency of protein utilization, it is known that individual AAs have different efficiencies of utilization (Aguilar et al., 1972); thus the AA profile of protein molecules consequently becomes an important factor to be consider when estimating efficiency of utilization. Conclusively, it was recognized that efficiency of AA utilization for gain varies depending on protein AA profile, animal maturity, energy intake, and daily gain (NRC, 1996; NRC, 2000; NASEM, 2016). Decreases in efficiency of AA utilization when protein is excessive are explained by antagonism among branched-chain AA, and additional energetic expenses associated with excretion of nitrogen (N) from excessive AA catabolism.

Differences between assays used to determine protein requirements also can create substantial discrepancies when comparing results from various studies. Stein et al. (2007) discussed differences among terminologies used to determine AA ileal digestibility (ID). Four distinct methods to measure AA digestibility are well characterized: total tract digestibility, apparent, true, or standardized ID. Each will differ depending on which variables are considered in calculations. Generally speaking, protein digestibility is calculated by the difference in AA intake and AA outflow. Total tract digestibility is estimated by subtracting fecal protein content from total protein intake; apparent AA ID is calculated by subtracting total ileal AA outflow from an animals AA intake; true ID corresponds to the fraction of dietary AA that is digested prior to the distal ileum; and standard ID is calculated in a manner similar to apparent ID, except that ileal endogenous AA losses are subtracted from ileal AA outflow (Stein et al., 2007).

Additionally, Titgemeyer (1997) identified other issues related to the design of nutrient digestibility studies. Differences among animal species (i.e. cattle or sheep), anatomical site of cannula (i.e. abomasum or proximal duodenum), type of cannula (i.e. T-type or reentrant), frequency/amount of feed intake, use of markers, digesta sampling method, study design, and method of data analysis were identified sources of variation. All these factors may impact conclusions and interpretations made regarding AA requirements and efficiency of utilization.

SUPPLEMENTATION OF NITROGENOUS COMPOUNDS TO RUMINANTS

Dietary Nitrogen Sources

When considering dietary protein sources for ruminant animals, all N-containing feed ingredients should be accounted for. Because ruminants host a complex microflora in their digestive tract, ruminants have the capacity to transform simple nitrogenous compounds (nonprotein N; NPN) into high quality protein to satisfy their requirements. Therefore, N sources used in ruminant diets can be classified in 3 categories: (1) NPN; (2) true ruminally-degradable protein (TRDP), and (3) ruminally-undegradable protein (RUP). The NASEM (2016), and previous editions, consider TRDP and NPN a single fraction of ruminally-degradable protein (RDP), where RDP and RUP are considered the two components of dietary feed crude protein (CP). True ruminally-degradable protein is referred to polypeptides, peptides, and free AA; while NPN is referred to ammonia and urea. Ammonia is used by ruminal bacteria for growth, and subsequently cells of dead bacteria are digested in the SI and used by the host as dietary protein. Microbial protein is the predominant source of AA absorbed in the SI of ruminants (NASEM, 2016).

The Cornell Net Carbohydrate Protein System (CNCPS; O'Connor et al., 1993) is a more complex scheme that divides CP into fractions based on the general mass action kinetics of all chemical reactions, where the rate of the reaction is dictated by the concentration of the reactants. Accordingly, the CNCPS divides CP into five fractions of N source (A, B_1, B_2, B_3, and C) which are correspondent to their rate of disappearance in the rumen (Sniffen et al., 1992). Each of these fractions will be identified and discussed throughout this section.

Non-protein Nitrogen

Fraction A is composed of NPN, which is expected to solubilize spontaneously in the rumen. In the NPN category, urea is the main ingredient used in ruminant diets. Crude protein content of feed stuffs is calculated by multiplying %N analyzed by 6.25; most protein sources are assumed to contain 16% N (Jones, 1931), which is the reason for multiplying %N by the nitrogen-to-protein conversion factor 6.25 (i.e. 100/16 = 6.25). Urea contains approximately 46% N, while most proteins average only 16% N, therefore, 1 kg of urea would provide 2.875 kg of CP equivalent (1 kg of protein would contain 0.16 x 6.25 = 1 kg of CP). In the rumen, urea and other NPN feed components are rapidly dissolved and hydrolyzed into ammonia by bacterial urease. Pearson and Smith (1943) demonstrated *in vitro* that ruminal fluid has such high urease activity that it would be nearly impossible to feed enough urea to saturate the reaction. Jin et al. (2017) studied the abundance of urease *ure*C gene in ruminal bacteria of dairy cows and concluded that the majority of ruminal urease-producing bacteria were still unidentified. Synthesis and activity of ruminal urease enzymes can be regulated by many factors, including concentrations of substrate (i.e. urea), end-product (i.e. ammonia), other dietary N sources (e.g. AA, amides), and ruminal pH, as discussed by Patra and Aschenbach (2018). Other nitrogenous compounds, such as ammonium salts, amides, amidines, biuret, and uric acid, were investigated

as NPN sources in ruminant diets (Hatfield et al., 1959; Oltjen et al., 1968); however, use of these ingredients as NPN sources in ruminant diets remain secondary to that of urea.

Dietary urea can be absorbed as urea, via urea transporters (Isozaki et al., 1994; Marini et al., 2004) or metabolized into ammonia. Ruminal ammonia can be absorbed as ammonia, utilized by ruminal bacteria to synthesize AA required for cellular growth, or it can bypass to the lower gut. In fact, Nolan and Leng (1972) estimated that approximately 80% of the N incorporated into microbial cells was derived from ammonia and only 20% was derived from free AA. A similar range (between 60 to 80%) of ammonia-N contribution to microbial protein was described by Pilgrim et al. (1970). Nonetheless, ruminal ammonia can disappear from the lumen of the rumen via three alternative pathways: assimilation into microbial cells, passage of digesta to the abomasum, or absorption across the rumen wall.

Early studies *in vitro* (Mooney and O'Donovan, 1970) and *in vivo* (Hogan, 1961; Bodeker et al., 1990) observed that ammonia absorption in the rumen was pH dependent, but the mechanisms of transport were not clear. Currently, it is recognized that transport of ammonia can occur via two different forms: (1) at pH > 7, NH_3 is transported in the lipophilic form, where rate of absorption is linearly corelated to the ruminal pH; while (2) at pH < 7, ammonia is primarily absorbed as NH_{4+} via putative K_+ channels in the apical membrane of ruminal epithelial cells (Abdoun et al., 2005)

Absorption of ammonia is correlated to ruminal pH; consequently, animal intoxication due to ammonia overload also is directly impacted by ruminal pH. Webb et al. (1972) demonstrated that ruminal ammonia-N concentrations can achieve values as great as 200 mg/100 mL without causing intoxication if ruminal pH is stable; however, if pH increases rapidly, such as due to a sudden increase in urea intake, ruminal ammonia-N can be toxic at concentrations

lower than 100 mg/100 mL. Ammonia intoxication commonly is associated with urea or ammonium salts overload, whereas poisoning from protein feeding is unlikely to occur. Because ammonia is mostly absorbed via facilitated diffusion at physiological pH (< 7.5), intoxication is improbable to occur when ruminal pH is below neutral. The membrane channel proteins responsible for NH_4^+ transport can become saturated, thus limiting uptake of ammonia via this route. On the other hand, when intraruminal pH is alkaline, ammonia is gaseous and mostly absorbed via lipid diffusion, becoming highly permeable through cell membranes via a transport mechanism that does not saturate. Abdoun et al. (2006) described the ruminal cell membrane as being about 175 times more permeable to NH_3 (gaseous form) than its ionized form, NH_4^+. Therefore, risk of ammonia intoxication increases as ruminal ammonia concentration increases, but it is when ruminal pH is above neutral that signs of toxicity are likely to be observed (Hogan, 1961; Abdoun et al., 2006). Excessive intake of urea increases ruminal pH and may be due to a variety of factors such as poorly mixed feed rations, incorrect diet formulation or adaptation, low water intake, poor-quality roughage.

High concentration of ammonia in the blood is extremely toxic to non-hepatic tissues, while also being permeable to the Blood-Brain Barrier. When circulating concentrations of ammonia exceed 0.8 mM, the animal may display signs of intoxication wherein cerebral damage can occur (Davidovich et al., 1977; Symonds et al., 1981). In the brain, ammonia is detoxified by glutamine synthetase enzyme, which uses ATP to catalyze the reaction between ammonia and glutamate to form glutamine. Acute intoxication with ammonia leads to a rapid cerebral increase of glutamine concentration (i.e., osmotically active molecule), associated with an ammonia-induced ATP depletion (Monfort et al., 2002). Accumulation of ammonia and glutamine, associated with ATP depletion in the brain, lead to functional disturbance of the central nervous

system, muscle tetany, and subsequent death. It is generally assumed that the liver efficiently metabolizes ammonia by converting it into urea or glutamine through the urea cycle; however, if hepatic capacity for ammonia detoxification is exceeded, peripheral ammonia levels will increase, leading to intoxication of the animal (Lewis et al., 1957).

Ruminally Degradable True Protein

Potentially degradable true protein is classified as fraction B by the CNCPS, which is sub-classified in 3 other levels according to their rate of degradation (Sniffen et al., 1992). Fraction B_1 has a greater rate of degradation and passage, while fraction B_3 has the slowest rate of disappearance, and fraction B_2 is classified as potentially degradable protein with an intermediate rate of degradation (Sniffen et al., 1992).

Bacteria attached to ruminal feed particles work symbiotically to promote effective protein degradation (Wallace, 1985). When considering the variety of chemical bonds present in a single protein molecule, it takes the synergistic functionality of a multitude of bacteria, with various specialized microbial proteases, to result in complete degradation of a large protein molecules (Brock et al., 1982). The final products of proteolysis, such as peptides and AA, are then transported inside microbial cells. Peptides can be incorporated into the bacterial cell or may be further degraded to AA. Depending on carbohydrate availability, intracellular AA can be incorporated into microbial cell protein for growth, or metabolized into VFA, CO_2, and ammonia to produce energy (Tamminga, 1979). Some ruminal bacteria have been described by Tamminga (1979) as incapable of excreting excess AA, thus needing to convert AA into ammonia in order to eliminate superfluous intracellular AA.

Bach et al. (2005) discussed in a review article some of the factors that can affect protein degradation in the rumen, such as type of protein (i.e., solubility characteristic), interactions with

other nutrients (i.e., Maillard reaction derivatives), the predominant microbial population, ruminal dilution rate (inversely correlated to protein degradation), and ruminal pH, where pH 5.5 to 7 is considered optimal for ruminal proteolytic enzymes.

Ruminally Undegradable Protein

The remaining C fraction is considered ruminally-undegradable, and includes protein fractions associated with polyphenolic compounds, heat-damaged proteins and ruminally-protected AA (Sniffen et al., 1992). These ruminally-undegradable protein represent an important source of AA to ruminants, second only to microbial protein (NRC, 1996).

Microbial Protein

Microbial protein can fulfill between 50% and 100% of the protein requirements for ruminants (NASEM, 2016). Shabi et al. (2000) partitioned AA flowing from the rumen to the abomasum as RUP, bacterial, protozoal, and endogenous protein fractions, and found that 33 and 11% of the CP flow were derived from bacteria and protozoa, respectively. Although, these values provide some estimate of bacterial and protozoal CP contribution to the SI, protein originating from microbial growth will vary considerably in response to available dietary energy. Synthesis of protein molecules requires a supply of energy for the establishment of peptide bonds. Generally, carbohydrates are used as the primary substrate for energy production in most biological reactions; however, additional dietary factors affecting efficiency of ruminal microbial protein synthesis have been extensively reviewed by many authors (Bergen and Yokoyama, 1977; Tamminga, 1979; Chalupa, 1980; Clark et al., 1992; Bach et al., 2005).

Because of this convoluted relationship between energy supply, rate of degradation and synthesis of protein in the rumen (i.e., ruminal protein may be catabolized for energy production, or microbial protein synthesis), researchers have tried to synchronize the supply of

protein and energy in the rumen in order to achieve better efficiency of N utilization (Herrera-Saldana et al., 1990; Henning et al., 1993). Although such synchronization of energy and N sources in the rumen is not yet practical to be applied in dietary formulations, it highlights the importance of supplying adequate amounts of available N when energy is not limiting. When carbohydrate fermentation is not sufficient to support protein degradation, N is lost as ammonia. Conversely, when availability of N is suboptimal in comparison to carbohydrate availability, microbial protein synthesis decreases, and in both cases, animal performance is not maximized (Nocek and Russell, 1988).

Antinutritional Factors and AA Profiles that Confer Resistance to Protein

Some feed proteins can be associated with antinutritional factors such as polyphenolic compounds, tannins, or lignin. Condensed tannins are protein binding agents that reduce digestibility by forming strong H bonds, thereby inhibiting activity of microbial digestive enzymes (Yacout, 2016). These binding complexes are mostly stable in a pH range between 3.5 and 7.0, but they dissociate at pH < 3.5 (Jones and Mangan, 1977). Thus, while they prevent protein degradation in the rumen, subsequent exposure to low pH in the abomasum would enable hydrolysis and absorption of AA in the SI (Waghorn and Shelton, 1997).

Other chemical bonds within protein chains have been identified as having negative effects on protein degradation. Hancock et al. (1994) described a positive relationship between the degree to which cysteine-disulfide bonds are cross-linked and resistance of the protein to ruminal proteolysis. Glycinin, and several leucine-containing peptides in the N-terminal group (commonly found in soybean meal), also are relatively resistant to degradation (Schwingel and Bates, 1996). Soybeans are known to contain protease inhibitors (i.e., trypsin inhibitors), although they are readily deactivated by heat treatment. Yang and Russell (1992) described

proline-containing peptides as having a slower degradation rate than other peptides, with Lys-proline dipeptides being degraded at least 5-fold slower than Lys-alanine dipeptides in the rumen.

Heat-damaged Proteins

Another factor that can result in resistance of proteins to degradation is heat treatment. By-product feed ingredients commonly used in cattle rations often are derived from manufacturing processes that employ heating. Heat damage is an important factor to be considered when evaluating protein digestibility and bioavailability.

Heat treated proteins have been shown to increase ruminal bypass protein (Cleale et al., 1987); however, bioavailability of this escape protein also may be decreased, which means it is absorbable but not necessarily suitable for metabolism in synthesis of protein (Gilani et al., 2012). Nakamura et al. (1994) observed that when protein was heat damaged, ruminal escape increased 8.5% on average in corn gluten meal and 4.5% in dried distillers' grains (DDG). Subsequently, Nakamura et al., (1994) evaluated bioavailability of escape protein based on animal performance, observing decreases in daily gain in response to heat damage of protein, leading the authors to conclude that absorbed N from heat-damaged protein sources may not be totally available for metabolism. Among all AA, lysine (Lys) is considered the most susceptible to heat damage during Maillard reactions, which significantly decreases its bioavailability (Erbersdobler and Hupe, 1991). Consequently, it is not surprising that in corn-based diets (Burris et al., 1976; Titgemeyer et al., 1988), rations with high inclusion of heat-damaged protein (e.g., DDG), even from sources other than corn, would also tend to have Lys as the first-limiting AA. Aside from the impact on Lys bioavailability, heat treatment has been described as one of the oldest and most common, methods for producing commercial bypass proteins (NRC, 2001).

Ruminally-protected AA

Multiple techniques have been proposed to promote AA protection from ruminal proteolysis (Broderick et al., 1991). Such protection usually is achieved through encapsulation to provide a protective coating around a purified AA core. To be useful for nutrition of ruminants, the encapsulation method must produce an AA that is resistant to degradation by ruminal microbes, but susceptible to post-ruminal digestion and absorption in the SI. Grass et al. (1972) developed a protective coat composed of a mixture of glyceryl tristearate and fatty acid that protected methionine (Met) from ruminal degradation, but release of Met in the SI was unsatisfactory. Innumerable alternative methods have been developed to promote ruminal bypass. Examples include: use of AA analogs and derivatives (Belasco, 1972; Amos et al., 1980), complexing with aldehydes (Hino and Russell, 1987), phosphonitrilic halides (Miller, 1972), acrolein acetals (Wildi and Miller, 1973), calcium soaps (Sklan, 1989), acetylenic esters (Wildi and Miller, 1973), alcohols, acids and alkalis (Schwab, 1995), and fatty acids (Baalsrud et al., 1976).

Lipid-encapsulated AA

Since the introduction of lipid-encapsulated AA, several products have been patented and introduced to the market. Because Met and Lys frequently are first-limiting AA in cattle diets, they are the most commonly encapsulated AA available commercially. The general method of oil and fat encapsulation is described by Yazawa et al. (1974); wherein a powdered form of the AA is mixed with a high melting point fat (> 40$_{o}$C) that keeps the AA of interest insoluble in the rumen. When the fat-coated AA reaches the SI, the capsule is susceptible to pancreatic lipolytic enzymes, which effectively digest the outer fat layer and release the AA core. The AA is then available for absorption in the SI of the animal (Yazawa et al, 1974).

Dietary triglycerides are metabolized rapidly within the rumen via lipolysis and biohydrogenation. Hydrolysis of triglycerides to yield glycerol and fatty acids proceeds relatively quickly in the rumen (NASEM, 2016). Unsaturated fatty acids, products of hydrolysis, are further converted to saturated fatty acids (SFA) via isomerization, followed by hydrogenation of the double bonds (reviewed by Jenkins et al., 2008). Most SFA, resulting from ruminal metabolization or directly from the diet, will leave the rumen to be degraded and absorbed in the SI. Because SFA are resistant to ruminal degradation and most bypass to the SI, they have been used as a physical protective barrier against ruminal microbial degradation for various dietary compounds (Voigt et al., 2006). Researchers have positively associated the degree of fat saturation with ruminal inertness (Zinn et al., 2000); while there is an inverse correlation to digestibility in the SI (Steele and Moore, 1968). Furthermore, encapsulation of AA with a lipid coating of SFA has been demonstrated to efficiently resist ruminal degradation, while the AA core is well degraded and available for absorption in the SI (Papas et al., 1984; Wu and Sandhu, 1986).

Polymeric coatings usually are pH-sensitive (Papas et al., 1984), and the acidity of cattle diets may impact the degree of protection provided by SFA and subsequent availability of the protected compound in the SI. Other characteristics such as composition, smoothness of the pellet surface, hardness of the inner core pellet, solubility of the ingredient, and pellet size also have been described as impacting efficacy of protective coating within the rumen (Wu and Papas, 1997). Ji et al. (2016) published a short communication that also described on-farm feeding practices that may impact efficacy of ruminally-protected AA products in ruminant diets; mechanical mixing was highlighted as a compromising practice that would impact efficacy of the protected product according to the encapsulation method applied.

PROTEIN DIGESTION IN RUMINANTS

Reticulorumen

The reticulum and the rumen are identified as two different compartments of the ruminant gastrointestinal tract, but because there is no sphincter between the two and their functions are not greatly distinct, they are often considered together (Dijkstra et al., 2005).

Dietary nutrients ingested by ruminants are first metabolized in the rumen by microbial populations. When dietary protein enters the rumen, bacteria and protozoa act synergistically to promote protein degradation (Hino and Russell, 1987). Hydrolysis of peptide bonds and deamination of AA have been identified as the key mechanisms by which protein is degraded anaerobically (Van Straalen and Tamminga, 1995). After a study performed by Chen et al. (1987) peptides were identified as being the main end products of protein degradation and peptide assimilation was determined to be a rate-limiting step in ruminal protein metabolism.

Although ruminal protozoa can hydrolyze protein extracellularly, their capacity to transport end products inside the cell and deaminate AA is limited (Forsberg et al., 1984). Ruminal bacteria can hydrolyze peptides, efficiently absorb AAs and peptides from surrounding medium, and deaminate AA (Hino and Russell, 1987). Peptides transported inside the bacterial cell can be utilized as peptides or are further broken down into AA that can be incorporated into bacterial cells, degraded into branched chain fatty acids, VFAs, ammonia, and CO_2 (Baldwin and Allison, 1983). Protozoa hydrolyze large protein molecules, while bacteria degrade and absorb small peptides and AA. Predation of bacteria allows ruminal protozoa to access AA and peptides for synthesis of protozoal protein, and when intracellular AA accumulate, they are excreted by protozoal cells where they become available for metabolism by bacteria.

Omasum and Abomasum

After the rumen, end products of fermentation such as ammonia, AA, VFA, and urea, plus endogenous protein, escape protein, and microbial protein, flow to the omasum and subsequently to the abomasum. The omasum functions as a contractile organ that reduces feed particle size as it passes to the abomasum (Becker et al., 1963). No enzymatic catabolic activity has been identified to take place in the omasum, but absorption of water, ammonia, VFA, and cations occurs to some extent (Johnston et al., 1961; Martens et al., 2004).

The abomasum is described as being equivalent to the monogastric stomach. Ruminal-escape protein, microbial protein, and endogenous protein are broken down into smaller molecules via secretion of gastric acid and proteolytic enzymes by specialized cells in the abomasum.

Small Intestine

From the abomasum forward in the digestion process, digestion in ruminants is very similar to that in monogastric animals. In the SI, partially digested polypeptides are cleaved to yield smaller protein molecules (i.e. AA, di- and tripeptides), which will be further absorbed in the brush border membrane of the enterocytes by specialized cell transporters.

Bicarbonate concentrations in pancreatic exocrine secretions are relatively low in ruminants compared to other species (Magee, 1961); consequently proteolysis occurs more posterior in the SI when compared to non-ruminants. This shift in site of protein digestion occurs to achieve optimum pH for enzymatic activity in the ruminant GIT. Ben-Ghedalia et al. (1974) observed that in sheep, trypsin, chymotrypsin and carboxypeptidase A become active only midway through the jejenum, while exopeptidase and dipeptidase were observed to become activate only after the first half of the ileum. As a consequence, the majority of AA absorption

occurs at middle to posterior portions of the ileum (Philips et al., 1976), with the greatest extent of absorption occurring at 7 to 15 m of distance from the pylorus (Ben-Ghedalia, et al., 1974).

Large Intestine

The large intestine (LI) of ruminants and monogastric animals, like the rumen, are colonized by a large population of microorganisms. An important function of the LI is the absorption of water. As in the rumen, products of microbial fermentation such as VFA, methane, ammonia, and microbial protein can be found (Williams, 1965; Murray et al., 1976). Energy availability for bacterial fermentation in the hindgut is critical and lesser than that of the rumen. As energy intake increases, more fermentable energy reaches the LI, thus leading to greater production of fermentation metabolites. Mason (1984) reviewed about metabolism of N compounds in the LI, and described that increasing LI fermentation activity leads to increased fecal N output, while urine-N excretion decreases. Nitrogen absorbed from the LI will only be advantageous to the ruminants if it is recycled back to the rumen (either as ammonia or urea) where ruminal microbes can use the N-source to produce microbial protein; otherwise, energy will be expended to excrete surplus N through urine.

Nitrogen Metabolism, Efficiency of Utilization, and Excretion

The majority of dietary N consumed by ruminants is absorbed as two main forms: ammonia-N (about 16 to 80%; Huntington, 1986), or amino acid-N (AA-N), with the proportions varying according to the nutrient profile of the diet. Ammonia produced in the rumen is derived from ruminal fermentation of both dietary and endogenous N sources, including urea. The extent to which N sources are degraded in the rumen depends on dietary characteristics, such as energy density, susceptibility to hydrolysis and AA composition. When AAs or peptides enter the blood, they can be used as protein building blocks or are catabolized to yield ammonia, which

subsequently is converted to urea. Urea has been identified as the most important end product of N metabolism in ruminants. From a quantitative perspective, approximately 70% of the N consumed daily passes through the bodily urea pool (Harmeyer and Martens, 1980). Additionally, recycling urea back to the rumen improves N utilization by making it available for microbial protein synthesis. Urea-N that is transformed into microbial protein-N can be further absorbed in the SI, thus providing to the host additional high-quality protein. About 40 to 80% of the urea produced in hepatocytes of ruminants can be recycled and excreted into the gastrointestinal tract via saliva or direct diffusion from blood, wherein rate of secretion has been described to be regulated by the concentration of blood urea (Harmeyer and Martens, 1980; Huntington, 1989).

Absorbed and metabolically produced ammonia is considered to be toxic to the animal, and hepatocytes have an important function in removal of ammonia from the blood by synthesizing urea, or on a smaller scale, glutamine, in order to remove all ammonia from circulation (Huntington and Reynolds, 1987). The liver accounts for no more than 2% of an animal's body tissue mass, but its intense metabolic activity can consume up to 26% of whole-body oxygen (Huntington and Reynolds, 1987). Such high oxygen demand is associated with energetic requirements to support (among all other metabolic processes) ureagenesis, protein synthesis and degradation, maintenance of ionic balance, substrate cycling, gluconeogenesis, and mixed-function oxidase activity (Huntington and McBride, 1988).

Lobley et al. (1996) infused multi-catheterized sheep with an isotope of ammonium chloride and observed that 59 to 67% of urea-N was derived from ammonia. Furthermore, the amounts varied depending on roughage:concentrate ratio of the diet. In a review article presented by Lapierre and Lobley (2001), rate of hepatic ureagenesis was positively correlated

with the N metabolites presented in the liver (i.e., ammonia-N vs. AA-N), and the energy intake level of the animal. Reynolds et al. (1991) reported that non-ammonia-N also can contribute to synthesis of urea in the liver. Overall, increases in dietary N load tend to increase production of endogenous urea. However, the degree of degradability and the site of degradation of the dietary N source, in combination with the absorption of degradation product (i.e., AA, dipeptides, urea, ammonia), are believed to be more closely corelated to hepatic ureagenesis activity (Huntington and Archibeque, 1999). Batista et al. (2016a) supplemented beef heifers with 0 or one of three levels of RUP to evaluate N metabolism and observed that as N intake increases (i.e., RUP supplementation), urea-N excretion, N retention, urea-N recycling, and microbial protein synthesis increases.

Other studies have focused on evaluating substrate priorities that determine the ratio of ammonia-N to AA-N for synthesis of urea. Lobley et al. (1998) infused an AA mixture into the mesenteric vein of sheep fed grass pellets and observed that hepatic synthesis of urea preferentially utilized ammonia-N over AA-N. Lobley et al. (1995) and Parker et al. (1995) implied that ammonia-N detoxification by the liver would require some input of other N sources, such as AA-N. Mutsvangwa et al. (1999), when working with *in vitro,* isolated sheep hepatocytes, suggested that ammonia-stimulated ureagenesis could result in Met catabolism, which would consequently compromise Met requirements of the animal. Reynolds (1992) also proposed that ammonia metabolism in the liver requires AA catabolism in order to form aspartate needed for ureagenesis. Later, Lobley et al. (2000) concluded that ammonia-N can contribute to both carbamoyl phosphate and aspartate-N inflow in the ornithine cycle. Although the increase in AA load tend to result in an increase in AA metabolization and urea production by the liver, Lobley et al. (2000), in agreement with Luo et al. (1995), stated that ammonia

detoxification in the liver can be effectively unassociated from AA oxidation according to the dietary profile.

Ideally, ruminant nutritionists expect that most AA-N are used for muscle tissue synthesis of beef production animals; however, hepatic N management and its partitioning between ammonia-N and AA-N to form urea was described to be a result of endocrine function (Reynolds et al., 1992), dietary N source (i.e., proportions of RDP, RUP and NPN), N load (Baptista et al., 2016a), and dietary energy supply (Lobley et al., 1995; Lobley et al., 1996). Collectively, these variables may concomitantly affect efficiency by which absorbed AA will be used by ruminant animals (i.e. tissue growth or ureagenesis). Among endocrine controlling factors, insulin and glucagon are considered the main regulators of nutrient utilization (Bassett, 1978). Nonetheless, other hormones such as catecholamines, cortisol, thyroid hormones, growth hormone, and the β-agonists (BAA), also can be involved in regulating nutrient utilization by the animal's body. Studies evaluating effects of BAA supplementation were in agreement that serum urea-N was decreased as lean tissue deposition was increased as a result of BAA supplementation (Kim et al., 1989; Walker and Drouillard, 2010; Parr et al., 2014). Aforementioned data led other authors to hypothesize that improvements in N utilization associated with BAA supplementation would shift cattle's growth curve to a greater capacity of muscle deposition, thereby increasing dietary CP requirements (Samuelson et al., 2017).

Beef production animals are supplemented with the objective to optimize animal growth, feed efficiency and consequently production profits. Proteinaceous ingredients are usually the most expensive dietary component of animal's diets. Nonetheless, the efficiency of which metabolizable protein is utilized by production animals are of great interest. The expectation is to optimize ruminal ammonia (from feed digestion or urea recycle) conversion to microbial

protein, and AA absorption in the SI. Additionally, in an ideal scenario, all absorbed AA would be destined for use in muscle accretion, thereby leading to greater production of animal protein; however, as previously mentioned, oxidative losses of plasma AA occur as a consequence of various physiological conditions and nutritional status of the animal. Lobley (1986) observed that efficiency of utilization of absorbed AA can vary from 37 to 80%. Protein synthesis and muscle mass maintenance are very active and energetically expensive. Thus, metabolizable energy must be available to maintain physiological processes and when in surplus of maintenance requirements, it allows protein deposition as muscle tissue. To maximize efficiency of AA utilization, AA catabolism within the body needs to be minimized. Lobley (2003) summarized AA catabolism as attending to three main routes of requirements: specific non-protein needs (transmethylation reaction involving Met), protein turnover, and AA surplus. Where AA surplus will occur either when absorbed AA exceed the capacity for protein deposition due to genetic or energetic restrictions, or due to an imbalanced ratio of dietary AA.

The urea produced as a result of hepatic N metabolism can be excreted via urine or re-cycled back to the GIT through saliva or diffusion from plasma to the rumen and other sections of the GIT (NASEM, 2016). Ruminal urea will be metabolized into ammonia and can either be used by ruminal microbes as a N source for synthesis of microbial protein or can be re-absorbed across the portal-drained viscera (NASEM, 2016).

To conclude, the complex intertwined N metabolism in ruminants has for over 50 years been a target for research aimed at improving animal performance. Optimizing N utilization by ruminants is of great interest of both livestock producers and environmentally-conscious consumers. Kebreab et al. (2001) studied the impact of N pollution by dairy cows and dietary alternatives to mitigate the issue, where reduction of superfluous N, especially of highly

degradable protein, was shown to reduce N excretion. Improvement in N utilization can lead to improvements in feed efficiency, increases in profitability, and reduction of environmental impact of animal husbandry by reducing N excretion by farm animals.

LYSINE REQUIREMENTS AND EFFICIENCY OF UTILIZATION IN RUMINANT ANIMALS

Determination of First Limiting AA and AA Requirements

A constant 50% efficiency of N utilization was once proposed for growing cattle (NRC, 1985). About one decade later, Ainslie et al. (1993) published data on conversion of metabolizable protein to net protein; thereafter, the NRC (1996) recognized that efficiencies of N utilization for gain would likely change as cattle mature. The 7th Revised Edition of the NRC (update 2000) assumed efficiency of N utilization as 49% for cattle at or above 300 kg; but also acknowledge that CP requirements may be less for cattle over 400 kg. Because the NRC (update 2000) conversion efficiency equation was developed based on data that only covered a weight range from 150 to 300 kg (Ainslie et al., 1993; Wilkerson et al., 1993), predictions outside this range may be considered unreliable. In the NASEM (2016), it was recognized that the protein models of previous editions could result in over- or underprediction of MP requirements for gain, thus an equation was proposed whereby efficiency of N utilization would be no less than 30%. The current and all previous prediction models assume efficiency of utilization as identical across all AAs, however, the NASEM (2016) recognizes the limitation of the protein model when it predicts all absorbed AA to be used with the same efficiency.

Predictions of AA requirements in ruminant species are somewhat complex in contrast to those of monogastric species. Alteration of dietary protein by ruminal microbes and synthesis of

microbial protein (which may account for 50% or more of MP) present major challenges for precisely defining requirements of ruminants. Nevertheless, researchers seeking for a better understanding about protein nutrition in ruminants have developed several techniques to improve the assessment of AA requirements. Richardson and Hatfield (1978) determined the first, second, and third-limiting AA on growing steers fed a semi-purified diet where microbial protein was the primary protein source. To evaluate the limiting AA for microbial protein, the authors supplemented individual AA and measured urinary N excretion, N retention, and plasma AA concentrations of growing steers. The authors concluded that Met was the first-limiting AA in microbial protein, followed by Lys and threonine as the second- and third-limiting AAs (Richardson and Hatfield, 1978).

Other researchers (Leibholz, 1975; Schwab et al., 1982; Abe et al., 1997; Abe et al., 1998; Abe et al., 1999) evaluated AA deficiencies in different diets fed to young calves. Until approximately 4 to 6 weeks of age, varying according to the dietary management (Beharka et al., 1998), young ruminants do not have significant development of the pre-gastric stomach compartments, and microbial protein is not considered to be a quantitatively important contributor in meeting the animal's requirement for protein. In this case, confounding factors resulting from fermentation in the rumen have smaller impact on estimations of AA deficiencies. Leibholz (1975) observed that Met was the first-limiting AA of post-weaned calves fed a starter ration based on barley and soybean meal. Similarly, Schwab et al. (1982) concluded that Met was the first-limiting AA for post-weaned calves fed a complete pelleted ration based on cereal by-product feeds. On the other hand, Abe et al. (1997) noted that post-weaned calves, when fed corn-based diets, would be limited first by Lys supply.

Along with aforementioned research, Foldager et al. (1977), Williams and Hewitt (1979) and Tzeng and Davis (1980) focused on quantifying the requirements for individual AA in pre-ruminant calves. Alternatively, cannulated animals voided of ruminal microbial populations and subjected to intragastric feed infusions have been used to estimate AA requirements in ruminants. In a like manner, this approach also aims to decrease microbial contributions and interferences when assessing ruminants' AA requirements. Fraser et al. (1991) infused lactating dairy cows with casein as a sole source of protein and identified Lys, Met, and histidine as the first-, second- and third-limiting AA of milk protein, respectively. When feeding soybean hull-based diets to Holstein steers, Greenwood and Titgemeyer (2000) identified Met, histidine, at least one of the branched-chain AA, and possibly Lys as limiting AA. Nolte et al. (2008) fed Rambouillet lambs diets with low RUP and infused intragastric mixtures of essential AA, and by using the deletion approach demonstrated that Met, threonine, arginine, tryptophan, and valine limited N retention. The deletion approach basically consists of supplying adequate or excessive amounts of all AA with the exception of one (Titgemeyer, 2003). Advantages and disadvantages of using each of the previous mentioned techniques to determine limiting AA and requirements in ruminants have been discussed in detail by Titgemeyer (2003).

Lysine Requirements and Efficiency of Utilization in Growing Cattle

The extent to which dietary AA lead to an increase in N retention by animals depend on a variety of factors such as AA composition of protein source, amount of NPN in the diet, energy density of the diet, percent of RUP in the diet, and AA profile and amount provided by ruminal microbial protein (Hill et al., 1980). Hence, a number of researchers have attempted to determine AA requirements of ruminants, but the extent to which experimental conditions modulate animals' requirements make their estimates somehow singular to the conditions

adopted by the author in the referenced experiment. Additionally, most experiments have been performed using cannulated animals that are housed in metabolic crates, and nutritionally maintained by intragastric infusions, where such experimental conditions tend to lead studied animals to perform below industry standards.

Williams and Smith (1975) conducted a study with cannulated, castrated male Friesian calves (110-160 kg), consuming a variety of diets, all of which were formulated to provide energy to support growth rates of about 0.4 kg/day. A series of infusions containing incremental amounts of Lys were performed through abomasal cannulas, and the corresponding plasma AA response curves were plotted to determine AA requirements of the animals. In this scenario, the authors estimated Lys requirement of castrated male calves as being less than 18.8 g/day (Williams and Smith; 1975). Williams and Hewitt (1979), later estimated Lys requirements of preruminant Friesian bull calves (50 to 58 kg) fed a synthetic milk-based diet as 7.8 g/day at a growth rate of 0.25 kg/day. Their estimates were much less than those described by Foldager et al. (1977), who estimated Lys requirements to be 12.15 g/day (0.78g/ kg $BW^{0.73}$) for 43-kg calves growing at a similar rate. When Merchen and Titgemeyer (1992) estimated Lys requirements from data reported by others, 16.52 g/day of Lys was estimated to be the need for maintenance of 250-kg steers and sustenance of 1 kg/day growth rate.

Fenderson and Bergen (1975) also conducted abomasal infusions in Holstein steers (274 kg) fed corn-based diets at 2.56 kg/100 kg BW daily. In their assessment of ruminants' essential AA requirements, Fenderson and Bergen (1975) estimated Lys requirements of growing steers as being not greater than 22.5 g/day. Observations of Burris et al. (1976), Hill et al. (1980), Greenwood and Titgemeyer (2000), and Klemesrud et al. (2000a) generally are in agreement with those of Fenderson and Bergen (1975) predictions, estimating values between 22 and 24.5

g/day for Lys requirements of growing steers. Batista et al. (2016a) performed abomasal infusions of incremental doses of Lys to assess efficiency of utilization and requirements of ruminally cannulated Holstein steers (165 kg). A breakpoint of 9 g/day of supplemental Lys was established, stating lack of advantage in N retention at greater levels of Lys supplementation. Their basal diet supplied 17.5 g/day of absorbable Lys, leading to an estimate of 26.6 g/day for the Lys requirements of growing steers (Batista et al., 2016a).

Little research is available on efficiencies with which individual AA are used by growing ruminants. The correlation between absorbed protein, deposited protein, and equivalent shrunk BW are factors used to determine efficiency of AA utilization by the CNCPS (O'Connor et al., 1993), where all essential AA are considered to be used with equal efficiencies. Gerrits et al. (1998) studied the efficiency of utilization of all essential AA from milk proteins by pre-ruminants Holstein calves, using protein deposition in response to increases in protein intake to estimate efficiency of utilization. Titgemeyer (2003), however, considered their efficiencies of AA utilization relatively low. It was pointed out that the marginal efficiencies (increases in AA deposition/increases in AA intake) are representative of minimum values for all essential AA except for the most limiting one. This is because AA intake (of all AAs besides the first-limiting one) will increase until it meets the requirement for the first-limiting AA. Batista et al. (2016b) performed continuous abomasal infusions of L-Lys and assessed urinary N excretion to determine N retention in ruminally cannulated Holstein steers (165 kg), and estimated efficiency of Lys utilization at 40% in growing steers. Using a similar approach, Hussein et al. (2016) evaluated the impact of ammonia load on efficiency of Lys utilization by cannulated Holstein steers (202 kg), and observed that ruminal ammonia load could improve efficiency of Lys

utilization by up to 18%, where steers receiving 0 g/day of urea had an efficiency of 51% for Lys utilization, while steers receiving 80 g/day of urea had an efficiency 69% for Lys utilization.

Based on previously reviewed data on efficiency of Lys utilization in growing cattle, it is unlikely that efficiency of utilization approaches the 100% assumed by Klemesrud et al. (2000a) when predicting metabolizable Lys requirements of growing cattle. Additional research is thus needed to clearly establish more accurate values of efficiency of Lys utilization in growing cattle.

Lysine Requirements and Efficiency of Utilization in Feedlot Cattle

Efficiency of AA utilization decreases as animal's weight increases (Ainslie et al., 1993). Amino acid requirements for growth are considered a function of animal's BW (Preston, 1966); and AA requirements increases as growth rate and/or energy density of the diet increases (Titgemeyer, 2003). The NRC (1996) predicts protein retention as being a product of weight gain (WG) and the protein profile of gain (PPG) (Protein retention = WG x 0.01 x PPG). Nonetheless, animals have not an endless capacity for protein deposition; each individual has a maximum genetic capacity depending on age, sex, live weight, and physiological state.

When production animals approach the finishing phase, they also advance to greater physiological maturity, thus growing at a slower rate compared to younger animals. Consequently, efficiency of AA utilization decreases as BW increases, while AA requirements for maintenance increase (MP = 4.1 x $BW_{0.5}$(kg) + 0.3 x $BW_{0.6}$ (kg) + [(DMI (kg) x 30 – 0.50 ((bacterial MP/0.8)-bacteria MP)] + endogenous MP/0.67); AA requirements for growth are calculated based on WG and thus decrease as ADG decreases (Net $Protein_{gain}$ = WG x (268 – (29.4 x (retained energy/ADG)));(NRC, 2001). The combination of AA requirements for growth

and maintenance forms the overall animals' AA requirements, which tend to plateau as they approach the finishing phase.

Klemesrud et al. (2000b) hypothesized that large-framed finishing calves may be deficient in metabolizable protein. Such a hypothesis was based on data from Sindt et al. (1993), whom observed that finishing calves supplemented with urea, blood meal, and feather meal were more efficient ($P < 0.05$) than calves supplement with just urea, during early stages of finishing. Ruminants fed corn-based diets were described to have growth rates limited by insufficient supply of Lys (Hill et al., 1980; Titgemeyer et al., 1988; Abe et al., 1997). Hence, Klemesrud et al. (2000b) conducted an experiment that evaluated effects of dietary metabolizable Lys on finishing cattle and observed improvement in feedlot performance of cattle supplemented with ruminally-protected Lys, especially during the early feeding period. Lysine requirements for 209-kg calves fed corn-based finishing diets and growing at a rate of 2.10 kg/day were estimated to be 40.5 g/day (Klemesrud et al., 2000b). In contrast, no growth responses were observed by Oke et al. (1986) when supplementing ruminally-protected Lys to 319-kg beef steers fed corn-based diets. Hussein and Berger (1995) evaluated the effects of supplementing ruminally-protected Lys and Met on performance of Holstein steers during the growing and finishing phases when fed whole-corn based diets containing soybean meal or soybean meal plus urea as supplemental N sources. No differences in daily gain or feed efficiency were observed, which led the authors to conclude that experimental diets were not limited in Lys or Met (Hussein and Berger, 1995).

The use of exogenous growth promoters, such as β-adrenergic agonists (BAA), is common practice in the beef cattle industry. Beta adrenergic agonists are approved to be fed continuously in a complete cattle feed or as a top-dress and are administered during the last 28 to

42 days on feed. The use of BAA has been described to improve feed conversion efficiency, rate of gain, carcass weight, and carcass red meat yield. These compounds repartition nutrients toward protein accretion, and also may decrease protein degradation and adipose tissue accretion. Results from Walter et al. (2016) illustrate that supplementation of BAA increased skeletal muscle protein and tended to increase N retention. The corresponding mode of action led Hosford et al. (2015) to hypothesize that steers fed zilpaterol hydrochloride (a type of BAA) would have greater requirements for metabolizable AA in order to achieve maximum performance. As Lys and Met were identified as common limiting AA for maximization of lean tissue growth, Hosford et al. (2015) conducted an experiment to evaluate the effects of supplementing BAA and ruminally-protected Lys and Met on finishing performance of feedlot cattle fed corn-based diets. It was observed that supplementation of Lys, Met, or Lys+Met during the period of BAA administration, and during the entire finishing period, improved efficiency of feed utilization (Hosford et al., 2015). Findings from Hosford et al. (2015) suggested that cattle fed zilpaterol may have greater metabolizable AA requirements to support maximum performance.

LIVER ABSCESSATION IN FEEDLOT CATTLE

Aggressive feeding programs such as those used by feedlots often are associated with increased incidence of liver abscesses in slaughtered cattle (Nagaraja and Chengappa, 1998). Data from Brown et al. (1973) and Brink et al. (1990) showed that liver abscesses reduced daily gains and feed efficiency of feedlot cattle. When comparing slaughtered cattle with severe liver abscesses versus healthy cattle or animals with less severe abscesses, Brown et al. (1975) reported that severe abscesses decrease gain and resulted in poorer dressing percentages

compared to animals with healthy livers. The rate of liver condemnations in U.S. cattle has been estimated to be 20.9%, where 13.7% were condemned due to some degree of abscessation (McKeith et al., 2012). A variety of dietary characteristics have been studied and associated with incidence of liver abscesses in feedlot cattle. Generally, the incidence is affected by amount and type of forage included in the finishing diets. Animals fed diets with greater levels of roughage were observed to have lower incidence of liver abscesses (Zinn and Plascencia, 1996). Utley et al. (1973), and Mader et al. (1993) observed that physical characteristics of the forage tended to impact incidence of liver abscesses. Additionally, the grain type also was described to affect incidence of liver abscess (Nagaraja and Lechtenberg, 2007), and incidence of liver abscesses typically is greater in feedlot steers than in heifers, which likely is due to differences in feed intake between heifers and steers. Holsteins, which normally are fed for longer periods than beef breeds, also have greater incidence of abscessed livers compared to slaughtered beef breeds.

Liver condemnations of slaughtered cattle represent major concerns for cattle producers and packers. Abscesses are a major cause of liver condemnations in packing plants, and the economic impact of liver condemnations is heavily influenced by suboptimal animal performance and decreased carcass yield compared to healthy cattle (Nagaraja and Chengappa, 1998).

Formation of liver abscesses in ruminants is considered secondary to ruminal lesions, often resultant from acidosis and ruminitis (Jensen et al., 1954). Hence, the same factors that lead to development of ruminal acidosis and ruminitis, such as irregular bunk management and abrupt changes in fermentability of the diet, can induce liver abscessation in cattle. Carcasses containing severe abscessed liver may require greater trimming due to migration of abscesses into surrounding tissues (Nagaraja and Lechtenberg, 2007). Furthermore, rupture of the

abscesses can lead to contamination of the carcass, which may cause extra trimming of the carcass, and interruption of the slaughter chain flow (Nagaraja and Chengappa, 1998).

Fusobacterium necrophorum

Fusobacterium necrophorum has been identified as the primary etiologic agent of liver abscesses in feedlot cattle (Nagaraja and Lechtenberg, 2007). *Fusobacterium necrophorum* is an anaerobic Gram-negative, nonmotile, nonsporulating, and rod-shaped (pleomorphic) bacterium. Members of the genera *Fusobacterium* are the most common anaerobes identified in animals with infectious pyonecrotic processes, where *F. necrophorum* is the most commonly isolated specie (Jang and Hirsh, 1994).

In the rumen, *Fusobacterium necrophorum* utilizes lactate as its primary energy substrate; some strains may ferment glucose, but generally *F. necrophorum* does not ferment carbohydrates (Tadepalli et al., 2009). Because *F. necrophorum* is a lactate-fermenting bacterium, its concentrations in the rumen tended to be 10-fold greater in grain-fed cattle than cattle fed forage-based diets (Tadepalli et al., 2009). Russell (2005) also observed that fusobacterium species isolated from the rumen could utilize Lys as an energy source for growth. In fact, the strains isolated from dairy cattle fed timothy hay or a commercial dairy ration showed very rapid rates of Lys degradation (Russell, 2005). Amino acids are degraded in the rumen by a group of bacteria generally classified as hyper-ammonia producing bacteria (Russell et al., 1991). Attwood et al. (1998) identified a strain of *F. necrophorum* isolated from the rumen, that is hyper-ammonia producing. Thereafter, Russel (2005) described *F. necrophorum's* great capacity for degrading Lys. Those observations led subsequent authors to hypothesized that *F. necrophorum* may be the major bacteria involved in Lys degradation within the rumen. Elwakeel et al. (2013) conducted *in vitro* studies to characterize Lys degradation by ruminal *F.*

necrophorum using seven ruminal isolates that were grown on lactate or Lys as the primary energy sources. Both sub-species of ruminal *F. necrophorum* (necrophorum and funduliforme) were described to use Lys as their primary carbon and energy source.

Outer membrane proteins are considered key factors to allow bacterial attachment and cause subsequent host infection (Pizarro-Cerdá and Cossart, 2006). Takayama et al. (2000) studied binding affinity of *F. necrophorum* to cells derived from inflamed ruminal epithelium and reported strong affinity to cells derived from ruminitis. The correlation between ruminal ulcerative lesions and incidence of liver abscess in feedlot cattle was first described by Smith (1944). Later, Jensen et al. (1954) conducted a study confirming this relationship and referred to the pathology as "ruminitis-liver abscess complex". In a review article, Nagaraja and Lechtenberg (2007) explained in great detail the pathogenic mechanisms that allow *F. necrophorum* to migrate from the rumen to the liver, and the subsequent formation of the abscesses in the organ.

Prophylaxis

The control of liver abscesses in feedlot cattle has been heavily dependent on the use of antimicrobial compounds. Antibacterial drugs are largely incorporated in feedlot diets to improve overall animal health and feed conversion (NASEM, 2016); however, the use of antibiotics in livestock production has become a matter of concern. Extensive use of low antimicrobial doses for growth promotion was identified as favoring the emergence of environmentally resistant bacteria, putting human society and other animals at risk (U.S. Food and Drug Administration [FDA], 2015). As a consequence, in June of 2015 the FDA announced the final rule that restricted access to these drugs and prohibited their use for growth promotion. In January 2017, the FDA Center for Veterinary Medicine (CVM) and animal drug

manufacturers completed the voluntary transition of all medically important antimicrobial drugs used in or on animal feed, from over-the-counter to Veterinary Feed Directive marketing status, thus requiring a veterinary prescription for their use.

Considerable efforts have been made to develop effective vaccines to prevent liver abscessation in cattle (Nagaraja and Chengappa, 1998). Fox et al. (2009) compared the efficacy of two commercially available vaccines: Fusogard® (Novartis Animal Health US, Inc, Greensboro, NC) and Centurion™ (Intervet/Schering-Plough Animal Health, DeSoto, KS), on reduction of liver abscesses in beef cattle, but no effect on incidence or severity of liver abscesses were observed.

Nutritional management can be just as crucial as a means of preventing liver abscess in cattle. Circumstances that lead to ruminal imbalance can damage the ruminal wall and favor the attachment with subsequent invasion of the pathogen in the host. Therefore, appropriate bunk management, which includes regular schedules and amounts of feed delivered, proper dietary adaptation to new rations, well mixed rations, and adequate access to feed and water, are some of the factors that can impact the rumen health in cattle. Also, significant effort has been made to identify alternative management strategies, feed ingredients, or non-antibiotic additives to mitigate incidence of liver abscesses in feedlot cattle (Narayanan et al., 2003; Checkley et al., 2005; Liu et al., 2010; Keele et al., 2016; Scott et al., 2017); however, experimental designs that aim to study preventive measures against liver abscesses are limited by the overall incidence of the lot in order to have statistical significance among treatments. If causational factors of liver abscess were identified, preventive or curative methods could perhaps be more quickly identified and compared. Gibb et al. (2004) and Mir et al. (2008) observed that sunflower seed supplementation to finishing steers reduced incidence and severity of liver abscesses. Others

described a quadratic decrease in incidence of liver abscesses when any amount of dried full-fat corn germ was included in finishing cattle diets (Montgomery et al., 2005). Decreases in liver abscesses were attributed to the decrease in starch or feed pattern intake caused by dried full-fat corn germ supplementation.

Based on the well-accepted pathogenesis of liver abscess in cattle, it is presumed that products that improve gut health may aid in preventing hepatic abscessation in cattle. Studies evaluating finishing performance in cattle fed ionophore antibiotics such as monensin, lasalocid, or laidlomycin propionate have shown no effect on incidence of liver abscess (Potter et al., 1976; Potter et al., 1985; Bauer et al., 1995; Depenbusch et al., 2008). Wagner et al. (2016) used meta-analytic methods to study effects of *Saccharomyces cerevisiae* fermentation products on finishing performance of beef cattle and concluded that there was a tendency to reduce liver abscesses rates when *Saccharomyces cerevisiae* fermentation products were supplemented. On the other hand, Scott et al. (2017) observed only a numerically decrease in incidence of liver abscesses when feedlot heifers were supplemented with a proprietary *Saccharomyces cerevisiae* fermentation prototype, without including monensin, tylosin, or other direct-fed microbials (DFM) in the diet.

Because liver abscess is well accepted as being a secondary pathological effect to a primary infection in the ruminal wall, such as ruminal acidosis and rumenitis, it is reasonable to speculate that the use of DFM that prevent those events could have some impact on incidence of liver abscess in cattle fed highly fermentable diets.

Megasphaera elsdenii

Megasphaera elsdenii is a lactate-utilizing bacterium, available commercially as an orally-drenched probiotic (strain NCIMB 41125; Lactipro Advance; MS Biotec LLC., Wamego,

KS). Lactate is an intermediate product of ruminal fermentation and it can be further metabolized to VFA or accumulate within the rumen. Insufficiency of lactate-utilizing bacteria can lead to accumulation of lactic acid which is injurious to the epithelium of the ruminal wall. The adaptation period intends to transition cattle from forage (slow rate and lower extent of fermentation) to high-grain diets (high rate and greater extent of fermentation), and is a key step to allow the rumen to be populated by lactate-utilizing bacteria (Counotte and Prins, 1981). *Megasphaera elsdenii* is an acid tolerant (Therion et al., 1982), Gram-negative coccus. This bacterium was considered by Counotte and Prins (1981) as the most important lactate-fermenting organism in the rumen, fermenting up to 80% of the lactate produced in the rumen of dairy cows; therefore, *M. elsdenii* is commonly regarded as a key microorganism in preventing ruminal lactate accumulation in grain-fed ruminants. If dosing cattle with *M. elsdenii* reduce incidence of ruminal acidosis, one may suspect that it also mitigates secondary conditions that result from inflammation of the ruminal wall, such as liver abscessation.

Ruminal concentrations of *F. necrophorum* increase as proportions of grain increase in cattle diets (Tan et al., 1994). As previously mentioned, *F. necrophorum* uses lactate as the major energy substrate and does not use carbohydrates. Greater abundance of the organism observed in grain-fed cattle likely is reflective of lactate availability. Thus, it is plausible to consider that cattle dosed with *M. elsdenii,* before transition to high-grain diets, have less incidence of ruminitis and acidosis, which would prevent the resulting secondary disorder: liver abscessation. Additionally, because these two microorganisms compete for lactate, increases in *M. elsdenii* populations within the rumen could competitively inhibit *F. necrophorum* as a consequence of substrate competition. *Megasphaera elsdenii* also is one of the few ruminal microbes that is able to survive and grow on AA when carbohydrates are limiting (Baldwin and

Allison, 1983). Although this hypothesis is well sustained, previous studies evaluating the effect of dosing cattle with *M. elsdenii* in finishing cattle performance have shown no impact on incidence or severity of liver abscesses (Leeuw et al., 2009; Drouillard et al., 2012; Ellerman et al., 2017; DeClerck et al., 2020).

CONCLUSION

Lysine is an essential AA present in low concentrations in corn-based diets, and is commonly identified as the first-limiting AA for backgrounding cattle. Supplementation of RPL to growing cattle has demonstrated improvements in growth performance. While finishing cattle are unlikely to have performance limited by dietary AA, physiological changes induced by supplementation of growth promoting agents, such as beta-agonists, could lead to an increase in protein requirements. There still are many questions surrounding advocacy for RPL supplementation, and studies described in this thesis focus on supplementation of RPL in corn-based diets at different stages of cattle growth.

There are characteristics of finishing diets that are associated with increased incidence of liver abscesses in cattle. Liver abscessation decreases feedlot performance and is linked with losses in overall carcass value due to condemnation of livers and trimming of carcass tissues. Identification of dietary factors that induce liver abscessation, and mitigating agents that could improve overall animal health, have the potential to improve feedlot performance, decrease antimicrobial usage, and increase overall carcass value of finishing cattle. Thus, dietary aspects of liver abscessation in feedlot cattle and alternative strategies intended to mitigate incidence and severity of abscesses were also investigated.

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Chapter 2 - Ruminally-protected lysine (SafeGain™) improves performance of growing beef cattle

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INTRODUCTION

Ruminal bacteria metabolize dietary nutrients prior to digestion in the small intestine of the host animal. Nitrogen-containing compounds enter the duodenum as microbial protein, protein from endogenous losses, or ruminally undegraded dietary protein. In many circumstances, ruminal degradation can be excessive, in which case host requirements for amino acids (AA) are not fully satisfied and their performance becomes sub-optimal.

Several methods have been identified to provide protective barriers against protein degradation in the rumen (Chalupa, 1975). Encapsulation methods have been used to increase post-ruminal supply of limiting AA (Papas et al., 1984), thus making it possible to more precisely meet animals requirements. Lysine (Lys) was identified as the first-limiting AA for growing cattle fed corn-based diets (Burris et al., 1976; Titgemeyer et al., 1988), and as the second-limiting AA when cattle are fed protein-free diets, in which case microbial cells become the primary protein source of metabolizable protein (Richardson and Hatfield, 1978). Thus, supplementation of Lys sources that resist microbial digestion, may, under some circumstances, provide an effective means for enhancing productivity of growing beef cattle.

Authors of previous studies have described improvements in performance when supplementing ruminally protected lysine (RPL) to growing cattle (Oke et al., 1986; Titgemeyer et al., 1988; Klemesrud et al., 2000a); however, differences in degree of protection of RPL source, basal diet, number of animals per pen, and average performance of control animals are variables that need to be considered when evaluating responses to Lys.

Thus, the objectives of this study were to evaluate the impact of RPL (SafeGain ™, H.J. Baker & Bro. LLC., Shelton, CT), an encapsulated form of Lys sulfate, on rate of body weight gain, daily feed intake, and efficiency of feed utilization in backgrounding crossbred beef heifers, as well as carryover effects during the subsequent finishing period.

MATERIALS AND METHODS

Procedures used during the live animal phase of this experiments were approved by the Kansas State University Institutional Animal Care and Use Committee. The live animal phase of the experiment was conducted at the Kansas State University Beef Cattle Research Center in Manhattan, KS. The laboratory phase was conducted at the Kansas State University Pre-Harvest Food Safety and Microbiology Laboratory, Manhattan, KS.

Experimental Design

A total of 448 crossbred beef heifers (287 ± 14.1 kg initial body weight) were selected from a lot of 690 animals and used to evaluate effects of RPL supplementation on feedlot performance of growing cattle. Heifers were weighed and weights were recorded in an Excel spreadsheet to allow stratification (based on initial body weight) with the SORT function of Excel. Of the original 690 animals, a group of 448 animals with the lowest standard deviation for initial body weight (BW) were enrolled into the experiment. Enrolled animals then were

stratified by BW to form weight blocks, and randomly assigned to 1 of the 64 partially-covered, concrete-surfaced pens (7 animals/pen). The study was conducted as a randomized complete block design with 4 dietary treatments and blocks. Treatments consisted of 0, 15, 30, or 45 grams/animal daily of RPL, which was incorporated directly into the total mixed rations.

Animal Processing

Upon arrival at the Kansas State University Beef Cattle Research Center, heifers were given *ad libitum* access to alfalfa hay and water and processed within 24 to 48 hours after arrival. Initial processing included individual identification of animals in the form of a numbered ear tag, recording of individual BW, vaccination against clostridial (Ultrabac 7/Somnubac, Zoetis Animal Health, Florham Park, NJ) and viral pathogens (Bovi-Shield Gold 5, Zoetis Animal Health), and application of parasiticide (StandGuard, Elanco Animal Health, Greenfield, IN). Animals were also evaluated for exclusion criteria, including visual symptoms of disease or injury, poor temperament, dairy breeds, and excessively heavy or light BW in comparison to their contemporaries.

On day 1 of the backgrounding phase, animals were implanted with Component TE-200 with Tylan (Elanco Animal Health), and initial BW was recorded as animals were sorted into designated study pens.

Diet Preparation, Sampling and Analysis

Composition of diets fed during the growing and finishing phases is shown in Table 2.1 and Table 2.2, respectively. Cattle were fed a corn-based diet during both phases. Ruminally-protected Lys was premixed into a vitamin and mineral supplement and fed to deliver 0; 0.1646; 0.3312; or 0.4958% of diet dry matter, or approximately 0, 15, 30, or 45 grams of Lys per animal

daily, respectively. Experimental diets were mixed and delivered once daily, between 1400 h and 1700 h, and cattle were allowed *ad libitum* access to feed and water for a period of 112 days.

Intakes were daily monitored, and daily feed deliveries were adjusted as needed to insure *ad libitum* access to diets. Feed bunks were managed to allow for 50 to 500 grams of residual feed dry matter per animal, immediately before next feeding period. Unconsumed feed, when excessive, was collected before daily feeding, weighed, sub-sampled, dried at 55ºC for 48 hours, and subtracted from the amount fed to each pen to estimate actual dry matter intake for each pen of cattle. Feed ingredients used in experimental rations were sampled upon arrival and weekly thereafter, dried at 55ºC for 48 hours, and composited into monthly samples which were later sent to a commercial laboratory (SDK Labs; Hutchinson, KS) for analyses of nutrient composition.

Ruminally-protected Lys used for this study was declared by manufacturer as containing approximately 30% L-lysine sulfate (H.J. Baker & Bro., LLC.). Nitrogen analysis (TruMac™ N Macro Determinator, Leco Corporation, St. Joseph, MI) were performed on RPL source, to determine N concentration of the product (average 7.96% nitrogen).

In Situ Determination of Ruminal Stability of Ruminally-Protected Lysine

Ruminal stability of RPL was determined via the *in situ* and results are illustrated in Figure 2.1. Three rumen-fistulated Jersey steers were used. Animals were housed in a concrete-surfaced pen with *ad libitum* access to water and feed. The basal diet was composed of 45% brome hay, 25.61% steam-flaked corn, 25% Sweet Bran® (Cargill Corn Milling, Blair, NE), and 4.39% of a vitamin-mineral supplement. The *in situ* technique was performed according to standard procedures suggested by Vanzant et al. (1998). *In situ* bags (R510; ANKOM Technology, Macedon, NY) were used to determine ruminal degradability (i.e., dry matter

disappearance and crude protein disappearance). One gram of RPL was weighed into the *in situ* bag (5 cm × 10 cm size and 50 μm porosity) and incubated into the rumen for 0, 8, or 16 hours. Each fistulated animal received two replicates of the sample (RPL or blank) and incubation time. After removed from the rumen, *in situ* bags were gently hand-washed in buckets where rinse water was changed until appears clear. Bags were gently squeezed to remove excess of water and then placed in to a 55 °C forced-air oven for at least 48 hours. At the end of the drying cycle, bags were removed from the oven and put into a desiccator to cool down. Residual content was weighed and percent dry matter disappearance (DMD) was estimated as follows:

$$\%DMD = 100*\left(1 - \frac{\text{Final DM} - \text{Initial Dry Bag Weight}}{\text{Initial DM}} * \text{Correction Factor}\right)$$

$$\text{Correction Factor} = \frac{\text{Final Blank Bag Dry Weight}}{\text{Initial Blank Bag Dry Weight}}$$

Residual matter was analyzed for protein content using a N analyzer (TruMac™ N Macro Determinator, Leco Corporation, St. Joseph, MI). Adjustment for ruminal bacteria contamination in the residual sample was not performed. Nitrogen content of Lys (encapsulated product) were assumed to be 19.16 %, thus protein content was estimated using N x 5.22 as conversion factor. Crude protein disappearance (CPD) was estimated as:

$$\%CPD = \frac{100 * ((((\text{InitialDM} * \%\text{InitialCP}) - ((\text{InitialDM} * (1- (\%DMD))) * (\%\ \text{ResidualCP})))}{(\text{InitialDM} * \text{InitialCP})}$$

Backgrounding Performance

On day 112, animals were weighed by pen using a large platform scale and final BW, average daily gain (ADG), dry matter intake (DMI), and feed efficiency (G:F) were calculated to access feedlot performance of each study pen during the growing phase. Subsequently, a subset of 48 of the originally 64 pens were selected to evaluate carryover effects of RPL fed during the growing phase on performance during the following finishing phase. Pens of cattle selected for the subsequent finishing phase consisted of the 48 growing pens with the smallest standard deviation with respect to body weight. Finishing diets were formulated to contain two different concentrations of antioxidants to assess impact on finishing performance, carcass traits, and incidence and severity of liver abscesses in feedlot heifers. No interactions between Lys fed during the backgrounding phase and antioxidants fed during the finishing phase were observed. Müller et al. (2018) described the effects of antioxidants on performance and carcass characteristics of finishing heifers; this paper will present only carryover effects of RPL fed during growing phase on performance during the finishing phase.

Finishing Phase

At the end of the backgrounding phase, cattle were placed onto a common diet and fed for 30 days before initiating the 21-day step-up period to transition to finishing phase. Two pens (7 animals/pen) of a given backgrounding phase treatment were consolidated to form a single 14-head pen during the finishing phase (n=28 pens during finishing phase). Cattle were re-implanted with Component TE-200 with Tylan (Elanco Animal Health). Pens from each of the 4 backgrounding treatments were equally distributed across the two finishing treatments (i.e., antioxidant concentration of diets). Cattle were placed into dirt-surfaced pens and fed their respective finishing diets. On day 95, animals were weighed by pen using a platform scale, immediately before transporting to a commercial abattoir in Lexington, NE (approximately 440

km). Final shrunk BW was calculated as gross BW x 0.96. Dressing percentage was determined by averaging hot carcass weight (HCW) within feedlot pen and dividing that value by final shrunk BW.

Data Collection

Cattle BW were measured on day 1, and on day 112 of the growing phase; and on day 1 (or day 140 from initiation of backgrounding phase) and day 95 (or day 240 from initiation of backgrounding phase). Carcass characteristics were collected on the day of slaughter by trained Kansas State University personnel, and included animal identification, harvest order, HCW, and liver abscess incidence and severity. Incidence and severity of liver abscesses were evaluated based on Brown et al. (1975) scoring system: score 0 was assigned to a healthy liver, with no abscesses; A_- to livers with one or two small abscesses or inactive scars; A_o to livers with one or two large abscesses or multiple small abscesses; and A_+ to livers with more than two large abscesses, and/or livers with adhered surrounding tissues. After a 36-hour refrigeration period, longissimus muscle (LM) area, 12_{th}-rib subcutaneous fat, marbling score, USDA Yield Grade, and USDA Quality Grade data were collected.

Statistical Analysis

Data were analyzed using SAS software, Version 9.4 (SAS Inst. Inc., Cary, NC). Feedlot performance during the growing phase and finishing phase (initial and final BW, ADG, DMI and G:F), and non-categorical carcass data (HCW, dressing percentage, LM area, 12_{th} rib fat thickness, marbling score, and overall yield grade) were analyzed using the MIXED procedure of SAS. Categorical carcass data (liver abscess incidence and severity, USDA Quality Grade and USDA Yield Grade) were analyzed using the GLIMMIX procedure. Statistical models included fixed effects of RPL, antioxidant level, and their interaction; random effect was

block; and experimental unit was pen. Interactions between RPL and antioxidant were not detected ($P > 0.10$), therefore only main effects of RPL are shown. Least-squares mean differences were compared using the PDIFF function of SAS, and orthogonal contrasts were used to test for linear and quadratic effects of RPL supplementation. Tendencies were declared when $0.05 < P \leq 0.10$ and significant effect of treatment was affirmed when $P \leq 0.05$.

Optimum level of RPL supplementation was calculated by regressing levels of RPL supplementation against carcass weight gain. The optimum was determined by solving for the first derivative of the second order polynomial equation.

RESULTS AND DISCUSSION

During the backgrounding phase, one heifer from the 30 gram/animal daily of RPL treatment was removed from study due to sudden death. During the finishing phase, four additional heifers were removed from study: one from 30 g/animal daily of RPL treatment due to physical injury; two from 45 grams/animal daily of RPL due to mortality; and one from the control group due to bovine respiratory disease. Animals were necropsied at the Kansas State University College of Veterinary Medicine, Manhattan, KS. Causes of death or removal were deemed unrelated to backgrounding or finishing treatments.

Backgrounding Phase

Data from the backgrounding phase are presented in Table 2.3. A linear effect of RPL was detected for all feedlot performance parameters. Daily dry matter intake (DMI) linearly decreased ($P = 0.04$) with incremental amounts of RPL in backgrounding diets, while average daily gain (ADG) linearly increased ($P = 0.05$). Consequently, feed efficiency (G:F) of growing heifers improved linearly ($P < 0.001$) with addition of RPL to backgrounding diets. Level 2

model in the Nutrient Requirements for Beef Cattle (National Academy of Science, Engineering, 2016) predicted the basal diet itself supplied 111% of Lys requirements, but Lys contents of ingredients used for this trial were not analyzed, and thus may have been less than predicted by the model. Alternatively, Lys requirements of growing cattle may be greater than predicted by the model. Because supplementation of RPL improved ADG of growing heifers, our data suggest that the basal diet failed to supply adequate amounts of Lys to growing beef heifers, thus Lys was the first-limiting AA for backgrounding cattle fed corn-based diets. Klopfenstein et al. (1985) described ruminal microbial protein as being insufficient to meet ruminants AA requirements during periods of fast growth, and supplementation of ruminally undegraded AA could be used as an alternative to achieve animal requirements. Titgemeyer et al. (1988) performed post-ruminal infusions of L-Lys to cannulated beef steers, and observed an increase in N retention, indicating that Lys was first-limiting when feeding corn-based diets. Klemesrud et al. (2000) subsequently performed a growing study with individually-fed beef steers that were limit-fed a corn-sorghum based diet and concluded that growing diets were insufficient to meet Lys requirements, as supplementation of protected Lys improved daily gain of feedlot growing steers. Our study findings are in agreement with results described by other authors (Burris et al., 1976; Richardson and Hatfield, 1978; Hill et al., 1980; Titgemeyer et al., 1988; Klemesrud et al., 2000a).

Titgemeyer et al. (1988) estimated Lys requirements for growing beef steers at 44 g/day when methionine (Met) was second-limiting and 48 g/day when Met requirements were achieved. Klemesrud et al. (2000) estimated Lys requirement for growing beef steers as being 22.5 g/day, or 5.7 % of metabolizable protein. This estimation was obtained from a concomitant supplementation with Met, which often is regarded as the second limiting AA for corn-based

diets and thus increases Lys requirement when supplemented concomitantly (Titgemeyer et al., 1988). Regression analysis of our data predicted an optimum carcass gain when 24.8 g of RPL (or 7.37 g of bypass Lys) were included in the diet and an optimum ADG of growing beef heifers when 30 g/day of RPL (or 8.91 g of bypass Lys) were supplemented. Nitrogen analyses were conducted with a N analyzer, which limited our knowledge with respect to the quality of the AA source, thus intestinal digestibility of the product was not evaluated. Previous research has described ileal digestibility of fat encapsulated Lys as ranging from 85 to 100% (Rossi et al., 2003).

Balancing diets on a basis of ruminally undegradable protein (RUP) and ileal digestibility has been proposed as a means of meeting metabolic demands of the animal. Previous work defined growing cattle Lys requirements on the basis of dietary metabolizable protein (MP; (Titgemeyer et al., 1988; Wilkerson et al., 1993; Greenwood and Titgemeyer, 2000; Klemesrud et al., 2000a; Batista et al., 2016b), and others on the basis of RUP (Titgemeyer et al., 1988). Estimations of cattle requirements on the basis of metabolizable Lys are considered the most accurate way of determining Lys bio-availability of feedstuff to meet metabolic requirements (Stein et al., 2007), but the complexity and cost of assays that estimate MP, in addition to specificity of the estimations for each feed ingredient, makes it impractical for application in formulating commercial diets. On the other hand, diet formulations that are based on estimated RUP values can add some precision when formulating ruminant diets. In the present study, backgrounding cattle fed corn-based diets responded favorably to supplementation of RPL.

Finishing Phase

There were no interactions detected between RPL fed during backgrounding phase and antioxidant treatment instituted during the finishing phase, thus only effects of RPL are discussed

in this paper. Finishing performance of heifers fed RPL during backgrounding phase is presented in Table 2.4. Supplementing RPL during the growing phase did not affect feed efficiency or daily gain during the subsequent finishing phase ($P > 0.05$). There was a tendency ($P = 0.07$) to increase intake during finishing phase when heifers were supplemented with some level of RPL during backgrounding phase. Improvements realized during the growing phase were maintained after feedlot finishing. Heifers fed RPL during backgrounding phase had greater final shrunk BW and HCW than heifers fed 0 grams/animal daily of RPL ($P < 0.01$). Carcass characteristics are presented in Table 2.5. No differences were observed between control and any level of RPL supplementation during backgrounding phase for dressing percentage, LM area, 12$_{th}$ rib fat thickness, marbling score, USDA Quality Grade, or USDA Yield Grade.

Liver abscess incidence and severity are shown in Table 2.6. No tylosin was included in finishing diets. Incidence of liver abscess were not different ($P > 0.05$) between control and heifers that received any level of supplemental RPL during the backgrounding phase. Within mild abscesses, heifers not supplemented with RPL during the backgrounding phase had greater incidence ($P = 0.05$) than heifers supplemented with any level of RPL, but no differences within the moderate or severe abscess classifications were detected among experimental groups ($P > 0.05$).

Previous studies have investigated effects of RPL supplementation on growing performance of cattle, using various experimental conditions. The current study described carryover effects of Lys supplementation offered during the growing phase on the subsequent finishing phase. Previous research on supplementation of RPL during growing phase has not described subsequent performance of cattle during feedlot phase. Our results showed that

growth improvements achieved during the backgrounding period were sustained during finishing phase, as heifers supplemented with RPL during growing phase had greater shrunk BW and HCW ($P < 0.01$) than control animals (0 grams/animal daily of RPL). No differences in other carcass traits were observed, however ($P > 0.05$). Hussein and Berger (1995) investigated feedlot performance of Holstein steers supplemented with four different levels of RPL and Met (RPLM; 0, 5, 10, or 15 g·heifer^{-1}·d^{-1}) during a growing-finishing trial over 266 days. In contrast to our results, no differences were observed in carcass characteristics of Holstein steers supplemented with RPLM, however a cubic response in DMI and feed efficiency was reported ($P < 0.10$) when increasing dietary levels of RPLM in the diet (Hussein and Berger, 1995). Oke et al. (1986) supplemented crossbred Angus steers with 4 concentrations of RPLM (0, 0.01, 0.06, 0.11% of RPL) during growing and finishing phases. The authors reported greater gain for steers supplemented with RPLM during the growing phase but not during the finishing phase. Improvements in gain achieved during the growing phase were sustained until the end of finishing phase and resulted in greater carcass weights. Similar to our findings, no other differences in carcass quality or yield grade were observed.

Compensatory growth is defined as an accelerated growth response that occurs after a period of dietary deprivation, such as metabolizable protein restriction (Bohman, 1955; Drouillard et al., 1991). The assumption that backgrounding steers fed corn-based diets have growth rates restricted by Lys levels of the diet leads to the assumption that compensatory growth could occur during subsequent finishing phase, when Lys requirements are satisfactory on corn-based diets (Oke et al., 1986; Hussein and Berger, 1995; Klemesrud et al., 2000a). Differences in body weight among backgrounding treatments were maintained during the finishing phase, suggesting that there was little or no compensation during the finishing phase.

IMPLICATIONS

The 8th Revised Edition of Nutrient Requirement for Beef Cattle underpredicted the response of backgrounding cattle to supplemental Lys. Lysine requirements of growing cattle may be greater than currently predicted. Ruminally protected Lys supplementation was effective for improving growing performance of beef heifers fed roughage-based diets and improvements achieved during backgrounding were sustained during the subsequent finishing phase.

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TABLES

Table 2.1. Composition of diets fed to heifers over a 112-day backgrounding period.

	Ruminally-protected lysine, g/day			
Ingredient, % DM	0	15	30	45
Brome hay, chopped	45.00	45.00	45.00	45.00
Steam-flaked corn	25.44	25.41	25.39	25.37
Sweet Bran[a]	25.00	25.00	25.00	25.00
Vitamin-mineral premix[b]	2.13	2.13	2.13	2.13
Feed-additive premix[c]	2.43	2.45	2.48	2.52
Nutrient composition[d] (DM basis)				
Crude protein, %	13.68	13.75	13.82	13.89
Non-protein nitrogen, %	1.45	1.45	1.45	1.45
NE_m, Mcal/kg	1.74	1.74	1.74	1.74
NE_g, Mcal/kg	1.12	1.12	1.12	1.12
Neutral detergent fiber, %	41.46	41.45	41.44	41.43
Lys[e], %	0.36	0.42	0.48	0.53
Ca, %	0.65	0.65	0.65	0.65
P, %	0.48	0.48	0.48	0.48
NaCl, %	0.25	0.25	0.25	0.25

[a] Wet corn gluten feed (Cargill Starches & Sweeteners North America, Minneapolis, MN).

[b] Consisted of limestone, salt, urea, trace mineral premix, vitamin A premix, and vitamin E premix, to provide (total diet dry matter) 0.10 mg/kg cobalt; 10 mg/kg copper; 0.60 mg/kg iodine; 60 mg/kg manganese; 0.15 mg/kg selenium; 60 mg/kg zinc; 2205 IU/kg vitamin A; and 15 IU/kg vitamin E.

[c] Formulated to provide 300 mg of monensin (Rumensin; Elanco Animal Health, Greenfield, IN); 0.40 mg of melengestrol acetate (Heifermaxx®; Elanco Animal Health); and 0, 15, 30, or 45 g of RPL (30 % L-lysine sulfate; SafeGain™; H.J. Baker & Bro., LLC., Shelton, CT) per animal daily.

[d] Analyzed by SDK Laboratories (Hutchinson, KS).

[e] Lysine content was estimated by the Nutrient Requirement of Beef Cattle (8th Revised Edition).

Table 2.2. Composition of diets fed to heifers over a 95-day finishing period

Ingredient, % DM	
Steam-flaked corn	60.10
Sweet Bran[a]	30.00
Corn silage	8.00
Limestone	1.49
Salt	0.30
Vitamin/mineral premix[b]	0.11
Nutrient composition[c] (DM basis)	
Crude protein, %	13.40
NE_m, Mcal/kg	2.11
NE_g, Mcal/kg	1.46
Neutral detergent fiber, %	19.58
Lys, %	0.44
Ca, %	0.69
P, %	0.48
K, %	0.70
NaCl, %	0.30

[a] Wet corn gluten feed (Cargill Starches & Sweeteners North America, Minneapolis, MN).

[b] Formulated to provide (total diet DM) 0.15 mg/kg cobalt; 10 mg/kg copper; 0.50 mg/kg iodine; 20 mg/kg manganese; 0.10 mg/kg selenium; 30 mg/kg zinc; 2200 IU/kg vitamin A; and 22 IU/kg vitamin E. Monensin (Rumensin; Elanco Animal Health, Greenfield, IN) was fed at the rate of 33 mg/kg of feed.

[c] Analyzed by SDK Laboratories (Hutchinson, KS).

Table 2.3. Effects of ruminally-protected lysine (RPL) on performance of heifers over a 112-day backgrounding period.

	RPL, g/day					Contrasts	
Item	0	15	30	45	SEM	Linear	Quad
Initial BW, kg	286.5	286.6	286.6	286.6	0.15	0.64	0.79
DMI, kg/day	9.59$_{a}$	9.55$_{a,b}$	9.46$_{a,b}$	9.12$_{b}$	0.163	0.04	0.43
ADG, kg	1.27$_{a}$	1.33$_{b}$	1.32$_{ab}$	1.33$_{b}$	0.052	0.05	0.20
G:F	0.1323$_{a}$	0.1397$_{b,c}$	0.1393$_{b}$	0.1444$_{c}$	0.0018	< 0.01	0.52

$_{a, b, c}$ Means with a common superscript letter are not different, $P > 0.05$.

Table 2.4. Carryover effects of ruminally-protected lysine (RPL) fed during the backgrounding phase on performance of heifers during the subsequent 95-day finishing phase.

	RPL, g/day					Contrasts	
Item[1]	0	15	30	45	SEM	Linear	Quadratic
Initial BW, kg	429[a]	436[b]	434[b]	436[b]	6.28	0.04	0.20
DMI, kg/day	10.15[a]	10.73[b]	10.61[b]	10.77[b]	0.317	0.07	0.31
ADG, kg	1.26[a]	1.34[b]	1.33[b]	1.30[a,b]	0.053	0.40	0.07
G:F	0.1212	0.1236	0.1263	0.1201	0.0031	0.96	0.15

[a, b, c] Means with a common superscript letter are not different, $P > 0.05$.

[1] BW: body weight; DMI: dry matter intake; ADG: average daily gain; and G:F as abbreviation for feed efficiency.

Table 2.5. Carryover effects on carcass characteristics of feedlot heifers supplemented with ruminally-protected lysine during backgrounding phase.

	Ruminally-protected lysine, g/day					Contrasts	
Item*	0	15	30	45	SEM	Linear	Quadratic
HCW, kg	372.0$_{a}$	379.6$_{b}$	379.2$_{b}$	375.1$_{ab}$	2.79	0.27	< 0.01
Dressing yield, %	62.65	62.47	62.74	62.36	0.469	0.67	0.76
LM area, cm^2	91.93	94.11	93.60	95.44	1.525	0.27	0.87
Fat thickness, cm	1.51	1.48	1.59	1.47	0.058	0.95	0.27
Marbling score†	497.26	507.45	500.82	526.55	16.904	0.11	0.49
USDA Prime, %	7.98	5.99	10.08	8.91	4.295	0.59	0.89
USDA Choice, %	4.38	5.01	4.42	5.08	6.685	0.71	0.48
USDA Select, %	12.78	20.58	15.72	13.53	5.404	0.87	0.17
USDA Sub-Select$_1$, %	8.35	8.31	3.79	7.06	3.906	0.47	0.52
USDA Yield Grade	2.54	2.47	2.62	2.45	0.125	0.75	0.55
Yield Grade 1, %	10.37	7.97	7.59	7.60	4.300	0.50	0.67
Yield Grade 2, %	36.72	44.60	35.36	48.57	6.586	0.24	0.60
Yield Grade 3, %	42.01	41.74	44.15	36.80	7.509	0.56	0.48
Yield Grade 4, %	3.14	3.56	3.16	3.59	4.464	0.93	0.68
Yield Grade 5, %	3.13	0.00	5.21	0.00	4.701	0.74	0.73

*LM: longissimus muscle; and HCW: hot carcass weight.

$_{a, b, c}$ Means with a common superscript letter are not different, $P > 0.05$.

†500 to 599 = Small degree of marbling; 600 to 699 = Modest degree of marbling.

$_1$Carcasses graded as USDA Standard, Commercial, Utility, or Cutter.

Table 2.6. Liver abscess incidence and severity of feedlot heifers supplemented with ruminally-protected lysine during the backgrounding phase.

	Ruminally-protected lysine, g/day					Contrasts	
Liver abscess[1], %	0	15	30	45	SEM	Linear	Quadratic
Total	31.79	22.62	24.06	15.90	6.641	0.20	0.91
A-	24.57[a]	16.66[a,b]	18.53[a,b]	12.15[b]	5.679	0.05	0.85
A0	6.39	3.57	4.64	2.39	3.209	0.25	0.89
A+	0.89	1.20	1.84	1.26	1.771	0.74	0.71

[1]Severity determined as described by Brown et al. (1975).

[a, b, c] Means with a common superscript letter are not different, $P > 0.05$.

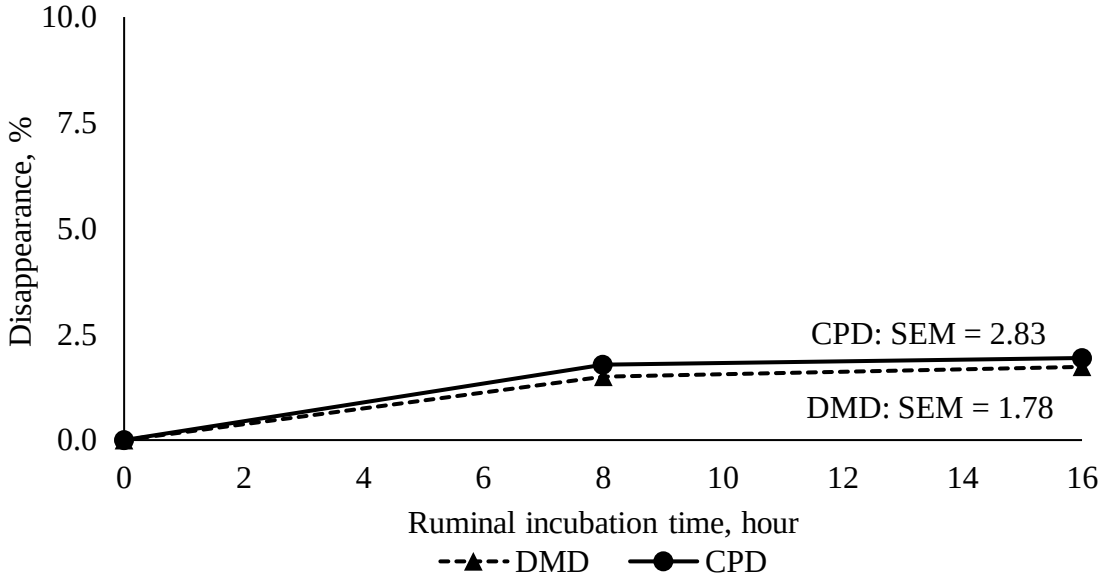

Figure 2.1. *In situ* determination of ruminal dry matter disappearance (DMD; •) and crude protein disappearance (CPD; ▲) of RPL source (SafeGain™; H.J. Baker & Bro. Inc., Shelton, CT).

Chapter 3 - Effect of ruminally-protected lysine on feedlot performance and carcass characteristics of finishing cattle

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INTRODUCTION

Ractopamine-HCl is a β-adrenergic agonist (BAA) that effectively redirects nutrients from fat deposition towards protein synthesis and muscle growth (Yang and McElligott, 1989; Watkins et al., 1990), and commonly is added to cattle finishing diets. Physiological conditions established by BAA administration led previous researchers to suggest that cattle fed finishing diets containing ractopamine-HCl would have greater requirements for dietary protein to sustain BAA-induced accretion of lean tissue (Samuelson et al., 2017).

The impact of feeding ractopamine-HCl on beef cattle is well understood and documented (Watkins et al., 1990; Dunshea et al., 1993; Quinn et al., 2008). In a monogastric digestive system, BAA administration can be directly correlated with changes in amino acid (AA) requirements; however, ruminal bacteria can degrade large portions of dietary protein, making uncertain the AA profile that becomes available for absorption in the gastrointestinal tract (GIT) of the host. Predicting AA requirements of ruminants treated with BAA agents is therefore challenging.

Lysine (Lys) has been described by previous studies, as being the first-limiting AA for growing cattle fed corn-based diets, which are rich in methionine (Met) supply (Burris et al.,

1976; Hill et al., 1980). Klemesrud et al. (2000) observed an improvement in average daily gain (ADG) during the first 56 days on feed when steers were fed corn-based finishing diets and supplemented with ruminally-protected Lys (RPL). Additionally, Batterham et al. (1990) and Apple et al. (2004) described enhancements in ADG and feed efficiency, when supplementing Lys to finishing pigs that were fed BAA. Aforementioned data suggest that finishing cattle fed BAA may have greater AA requirements to sustain additional lean tissue growth, and with Lys most likely being the first-limiting AA. We hypothesized that performance of finishing cattle fed corn-based diets could be improved by supplementing RPL in conjunction with BAA, and the objective of this study was therefore to evaluate the effects of supplementing RPL during the period of BAA administration in finishing beef steers.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocol and all procedures used in the current experiment. The live phase was conducted at the Kansas State University Beef Cattle Research Center in Manhattan, KS. Laboratory analysis were conducted at the Kansas State University Pre-Harvest Food Safety and Microbiology Laboratory, Manhattan, KS.

Animal Processing and Handling

A feedlot trial was conducted to evaluate effects of supplementing RPL on dry matter intake (DMI), daily body weight gain, feed efficiency (G:F), and carcass characteristics of finishing cattle. Four hundred thirty-one crossbred steers (413 ± 29.2 kg initial BW) were received at the Kansas State University Beef Cattle Research Center. Upon arrival, steers were offered *ad libitum* access to alfalfa hay and water before initial processing. Twenty-four hours

after arrival, steers were processed through the working facility and initial processing included measurements of body weight (BW), identification with an uniquely numbered ear tag, treatment for external parasites using StandGuard (Elanco Animal Health, Greenfield, IN), treatment for internal parasites with SafeGuard oral de-wormer (Merck Animal Health, Summit, NJ), vaccination against viral (Bovi-Shield Gold 5, Zoetis Inc., Florham Park, NJ) and clostridial (Ultrabac 7, Zoetis Inc.) pathogens. Cattle observed with signs of lameness, respiratory dysfunction, or other abnormalities at initial processing were removed from the initial experimental sorting group.

Experimental design

A subset of 384 crossbred beef steers (413 ± 29.2 kg initial BW) were selected from the original group of 431 animals and used to evaluate effect of supplementation of RPL, in conjunction with BAA, during the last 42 days of feedlot finishing. Steers were weighed and weights were plot into an Excel spreadsheet to allow stratification with the SORT function of Excel. The 384 animals used for this study consisted of the eligible animals that represented the lowest standard deviation for initial BW. Then, steers were stratified by BW, blocked by BW, and randomly assigned, within block, to 1 of the 64 study pens. On day 1 of finishing trial, animals were re-weighed during sorting process, and implanted with Component® TE-200 (Elanco Animal Health, Greenfield, IN), and placed into pens of 6 animals each, providing approximately 0.7 m of bunk space per animal. Study pens were partially-covered, concrete-surfaced, and equipped with automatic waterers between feed bunks that allowed *ad libitum* access to water. The study was conducted as a randomized complete block design with 2 x 4 factorial treatment arrangement and 8 replications per treatment. Factors consisted of the amount of RPL fed (0, 20, 40, or 60 grams/animal daily; SafeGain™, H. J. Baker & Bro. LLC., Shelton,

CT) during the final 42 days on feed (during the period of BAA administration; Optaflexx, Elanco Animal Health). Amounts of RPL composed approximately 0, 0.2040, 0.4163, or 0.6203% of the diet dry matter basis, respectively. The second factor was comprised of step-up regimen: a conventional 21-day adaptation period without *Megasphaera elsdenii* (-ME; Lactipro® NXT MS Biotec, Wamego, KS), or an accelerated 12-day step-up period with ME (+ME). *Megasphaera elsdenii* (ME) is a lactate-utilizing bacterium available in the market as a probiotic oral drench. Freeze-dried ME was re-hydrated and dosed orally (1×10^{10} CFU/steer) on day 1 of the trial to +ME treatment group. Both step-up regimens were composed of four intermediate rations until complete transition to finishing diets.

Sixty days before slaughter, steers were re-implanted with Component® TE-200 with Tylan (Elanco Animal Health). Final BW were determined immediately before transporting to a commercial abattoir for harvest (Lexington, NE), and ADG, DMI, and G:F were calculated as measurements of feedlot performance.

Feeding and Management

Steers were fed finishing diets once daily, *ad libitum*, for a total of 112 days. Finishing diets were composed of (dry basis) 68.87% steam-flaked corn, 20% Sweet Bran® (Cargill Corn Milling, Blair, NE), 7% alfalfa hay, and a vitamin and mineral premix (Table 3.1). The -ME and +ME treatment groups were fed a common finishing diet after completion of the step-up regimens until initiation of the BAA administration period. Ruminally-protected Lys was fed only during the period of BAA administration (the final 42 days on feed). Optaflexx (Elanco Animal Health) was fed to provide 300 mg/animal daily of active ingredient (ractopamine-HCl).

Bunks were scored one to two hours before feeding to determine amounts of feed to be delivered to each pen daily. The bunk scoring system intended to provide *ad libitum* access to

feed with minimal accumulation or unconsumed feed in the bunk (no more than approximately 500 grams per animal daily). In order to estimate daily intake, unconsumed feed was removed from feed bunk, weighed, and analyzed for dry matter content. Dry matter contents of refused feed samples were determined by drying samples in a 55°C forced-air oven for 48 hours. Total daily amount of feed delivered (as-fed basis) to each pen was recorded and dry matter consumption (DMI) per animal within pen was computed (per day) as:

$$\text{DMI} = \frac{((\text{Total feed offered}) \times (\%\text{DM})) - ((\text{Total feed refused}) \times (\%\text{DM}))}{\text{No. animals} \times \text{days}}$$

Corn was steam flaked daily, in the morning, to provide amounts needed for daily use in experimental diets. The grain was processed to a density of 360 g/L and final moisture content of 18.5%. Steam-flaked corn was sampled daily and analyzed for DM content (forced-air oven at 105°C for 24 hours) and starch availability. Diets were mixed using a truck-mounted feed mixer (model 490-14 Roto Mix; Dodge City, KS). Mixed diets were discharged into plastic barrels and hand delivered once daily to each experimental pen. Optaflexx and RPL were premixed and incorporated directly into the total mixed diet. The mixer was cleaned thoroughly between diets to avoid cross-contamination among treatments.

Diet sampling and analysis

Feed samples of ingredients used in diets were collected weekly, and upon receiving, stored in plastic bags, and conserved frozen. At the end of the trial, weekly samples were composited into monthly samples, which were then shipped to the SDK Laboratories (Hutchinson, KS) for analysis of nutrient composition of each feed ingredient. Results from each month were averaged to estimate final composition of each diet (Table 3.3) used during the experimental feeding period.

Data Collection

Cattle BW were measured within pen on day 1, and on day 112 of the finishing phase. Feedlot performance was evaluated based of ADG, DMI and G:F. At the end of the assigned feeding period, animals were shipped to a commercial abattoir (Tyson Fresh Meats, Lexington, NE). Carcass characteristics were collected the day of slaughter by trained Kansas State University personnel, and included animal identification, harvest order, hot carcass weights, and incidence and severity of liver abscesses. Liver abscess incidence and severity were evaluated as described by Brown et al. (1975), where score 0 was attributed to livers with no abscess, A^- to a mild abscessed liver (one or two small abscesses or with inactive scars), A^0 to a moderate abscessed liver (with one or two large abscesses or multiple small abscesses), or A^+ to a severely abscessed liver, defined as containing various large abscesses, with inflammation surrounding the abscess, and often seen adhered to adjacent tissue. Thirty-two hours after refrigeration, marbling score, 12^{th}-rib fat thickness, ribeye area, and USDA Yield Grade were obtained from camera images; and USDA Quality Grade, and incidence and severity of dark cutting beef were determined by a USDA grader.

Statistical Analyses

Data were analyzed using SAS software, Version 9.3 (SAS Inst. Inc., Cary, NC). Initial BW, ADG, DMI, G:F, and non-categorical carcass data (HCW, dressing percentage, Longissimus muscle (LM) area, 12^{th} rib fat thickness, marbling score, and overall yield grade) were analyzed using the MIXED procedure of SAS; while categorical carcass data (liver abscess incidence and severity, USDA Quality Grade, and USDA Yield Grade) were analyzed with the GLIMMIX procedure. Statistical model included fixed effects of RPL levels, ME, and potential two-way interactions; random effect was block; and experimental unit was pen. Least-squares

mean differences were compered using the PDIFF function of SAS and orthogonal contrasts were used to test for linear and quadratic effects of RPL supplementation. When $0.05 < P \leq 0.10$, a tendency for treatment effect was declared, whereas effect of treatment was only declared when $P \leq 0.05$.

RESULTS AND DISCUSSION

Feedlot Performance

No interactions were detected between RPL and ME for feedlot-performance indicators during the 112-day feeding period, therefore main effects of RPL and ME are reported separately. No impact of RPL supplementation during BAA administration period was observed for the overall feeding period (112 day; Table 3.2). Average daily gain, DMI, and G:F during feedlot finishing were not different between steers fed control diets (no supplemental RPL) and those supplemented with RPL during BAA administration ($P \geq 0.45$; Table 3.2). The current study observations support observations of Oke et al. (1986), whom found no advantageous effect of RPL supplementation on performance of finishing steers (368 kg initial BW). They attributed the lack of response to low protein requirements of finishing animals compared to growing steers. Additionally, Klemesrud et al. (2000) observed no advantages in supplementing RPL for a 161-day feeding period on performance of finishing calves (averaging 237 kg initial BW); although an increase in ADG and G:F during the first 56 days on feed was reported in response to 3 and 4 g/d RPL supplementation. It may be worthwhile to note that the study conducted by Klemesrud et al. (2000) preceded commercial availability of BAA, which could affect protein deposition patterns. Samuelson et al. (2007) observed no benefits from supplementing additional crude protein during the BAA administration period, leading the

authors to concluded that BAA supplementation did not increase protein requirements of finishing cattle. On the other hand, Hosford et al. (2015) evaluated the effect of supplemental Lys with zilpaterol-HCl on feedlot performance of finishing steers (averaging 366 kg initial BW) and observed improvements in ADG and feed conversion when RPL was top-dressed on finishing rations during BAA administration period (last 20 days on feed). Similarly to Klemesrud et al. (2000), but differently from the current data, RPL was supplemented during the entire 134-day finishing period of the study done by Hosford et al. (2015). The absence of a response to protein or AA supplementation during periods of BAA administration does not rule out the possibility that animal requirements increase in response to BAA. Protein generally is fed at concentrations deemed necessary to optimize ruminal energy metabolism, and it is likely that metabolizable protein supply is well in excess of actual requirements, so additional supply of metabolizable protein or AA yields no further benefit.

When evaluating response performance of cattle supplemented with ruminally-protected products, intestinal availability of the product must be considered. The intestinal availability of RPL (SafeGain, H.J. Baker & Bro., LLC.) used for the current study was not determined, but *in situ* incubations suggest that the RPL used in this experiment has a relatively low ruminal degradation (97% by-pass; later discussed in Chapter 4) delivering approximately 0, 6.61, 13.48, and 20.09 grams per day of Lys to the SI (assuming average DMI as 2% of BW). Hosford et al. (2015) top-dressed RPL to supply 12 g/animal daily of Lys to the SI, and concluded that cattle fed BAA may require additional Lys supplementation in order to maximize daily gain. Observations from our study failed to corroborate these findings. Klemesrud et al. (2000) supplemented incremental amounts of RPL to deliver 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 12 grams of Lys per animal daily to the SI; describing some advantages during first 56 days on feed by

supplementing RPL to finishing calves. Klemesrud et al. (2000) used young calves that were placed on feed directly after weaning, and metabolizable AA requirements likely are different from those mature animals most commonly introduced in feedlot finishing programs.

Cattle dosed with *M. elsdenii* were transitioned using an accelerated step-up regimen of a 12-day period without having negative impact on gut health. Brown et al. (2006) reported that adapting cattle within less than 14 days to high concentrate diets would negatively impact performance when cattle have *ad libitum* access to feed. Such reduction in performance would likely be attributed to GIT disturbances (e.g., acidosis) during the accelerated transition period. Nonetheless, Miller (2013) reported that although heifers dosed with ME and transitioned with an accelerated step-up regimen had lower performance during the step-up period, the overall performed was similar to that of their counterparties transitioned to high-concentrate diets using a traditional step-up approach. Additionally, the author pointed out that if acute acidosis had been a problem during the step-up period, a decrease in performance likely would have been observed. In the current study, cattle dosed with ME and transitioned via an accelerated step-up regimen also had performance similar to that of traditionally adapted cattle (Table 3.3). Use of an accelerated regimen simplifies logistics of feedlot operations, but also reduces the amount of roughage used in finishing cattle. Miller (2013) observed in a second study that roughage consumed during the step-up period accounted for 24 and 9% of the total roughage consumed during the study period for Control and ME-dosed steers, respectively.

Ractopamine-HCl redirects nutrients from fat deposition to protein synthesis and muscle growth (Yang and McElligott, 1989; Watkins et al., 1990). This mode of action lead previous researches to hypothesize that BAA administration may increase AA requirements of cattle (Hosford et al., 2015; Samuelson et al., 2017). Presumably, supplementation of RPL during the

period of BAA administration could potentially improve growth responses; however, this hypothesis was not supported by the current study. Increases in supplemental amounts of specific limiting AAs in feedlot diets (i.e., Lys) is one approach to optimize tissue growth; however, when the amount of AA supply exceeds animal's requirements for maximal body protein deposition, it may impair conversion of metabolizable protein to tissue protein due to energy expenses from metabolism and excretion of excessive nitrogen supply (NASEM, 2016).

Carcass Characteristics

Step-up regimen with or without ME and RPL supplementation showed an interaction in HCW of finishing steers ($P = 0.01$; Figure 3.1). When cattle were dosed with ME and adapted over a 12-day period, HCW was observed to be greater than control (i.e., no ME and traditionally transitioned over a 21-day period) when supplementing 20 grams of RPL per animal daily; but less than control when supplementing 0, 40 or 60 grams of RPL per animal daily. Oppositely, cattle not dosed with ME and traditionally transitioned to finishing diets over a 21-day period, presented lower average HCW than dosed animals (i.e., +ME) when supplemented with 20 grams of RPL/animal daily; but had greater average HCW when 0, 40 or 60 grams of RPL was supplemented. Baldwin and Allison (1983) reported that *M. elsdenii* is one of the few ruminal microbes that is able to not just survive, but also grow on AA, when carbohydrate availability is limited. Such characteristic of this microbe may explain the observed interaction between the RPL supplementation during BAA administration period and ME.

No interactions between RPL and ME were observed for other carcass characteristics, therefore only main effects of ME (Tables 3.4) and RPL (Table 3.5) will be presented.

Cattle dosed with ME and transitioned to finishing diets over a 12-day period had greater longissimus muscle (LM) area ($P = 0.04$), lower marbling scores ($P = 0.03$), and a higher

percentage of carcasses graded Select ($P < 0.03$) when comparing to control groups traditionally transitioned over a 21-day period (-ME). Our observations disagree with that of Miller (2013) who described a tendency for ME dosed cattle to grade more Choice ($P = 0.07$), while traditionally adapted cattle showed a tendency to grade more Select ($P = 0.06$). Other carcass characteristics as liver abscess incidence, and severity, 12th-rib fat thickness, and USDA Yield Grade were not affected by ME administration and transition regimen ($P > 0.21$).

Ruminally-protected Lys supplementation during BAA administration period showed a tendency to increase percentage of carcasses graded Select ($P = 0.07$) and tended to decrease percentage of carcasses graded Prime ($P = 0.08$). No other effects of RPL supplementation during BAA administration period were observed for marbling score, LM area, 12th rib fat thickness or USDA Yield Grade ($P > 0.18$). Klemesrud et al. (2000) reported no differences in HCW and USDA Quality Grade but pointed out a numerical advantage in HCW of finishing calves when supplementing 3 to 4 grams/animal daily of RPL during feedlot phase.

Incidence and severity of liver abscesses in finishing steers supplemented with RPL during BAA administration period is illustrated in Figure 3.2. A tendency to increase incidence of liver abscesses was observed with incremental amounts of RPL added into finishing diets during BAA administration period ($P = 0.10$). When contrasting control animals versus animals supplemented with any level of RPL during BAA administration period, an increase in incidence of liver abscesses was observed ($P = 0.04$). Additionally, there was a tendency for RPL to increase the severity of liver abscess in finishing cattle ($P = 0.07$), with animals fed greater amounts of RPL per day (i.e., 60 grams/animals daily) having greater percent of severely abscessed livers.

Incremental amounts of RPL into finishing diets, particularly used on this study as 20, 40 or 60 grams of RPL per animal daily, resulted in a numerical increase in BW and an opposite numerical decrease in HCW. This inverse relationship between live weight and carcass weight may be explained by the higher rate of liver abscess incidence and degree of severity presented by groups supplemented with any level of RPL versus control groups fed no supplemental RPL. The lighter carcass weights presented by heavier animals may be attributed to carcass trims due to adhesion of moderate to severely abscessed livers in the intrathoracic wall. Increase in liver abscess incidence and severity observed in this study suggests that RPL may stimulate liver abscesses formation in feedlot cattle.

Fusobacterium necrophorum is the predominant microorganisms associated with liver abscesses in cattle fed high-concentrate diets. Narayanan et al. (1997) and Tadepalli et al. (2009) provided support to the rumenitis-liver abscess complex hypothesis, when reported genetic similarities between *F. necrophorum* isolated from the abscessed liver and from the ruminal wall of slaughtered cattle. When the rumen wall is damaged (e.g., acidic pH or foreign objects) the ruminal epithelium can ulcerate which consequently allows *F. necrophorum* to penetrate the tissue and colonize the ruminal wall. Nagaraja & Chengappa (1998) described that once *F. necrophorum* colonizes the ruminal wall, it can subsequently release bacterial emboli into the portal blood, mechanism of which *F. necrophorum* utilizes to migrate to the liver, causing the resulting abscessation. Factors that perhaps favor pathogenic mechanism of *F. necrophorum* or its multiplication in the rumen could lead to an increase in incidence of liver abscesses in cattle.

High concentrate diets, such as those commonly fed to feedlot cattle, generate high yield of VFAs, which decreases ruminal pH, and can possibly lead to ruminal acidosis (pH < 5.5). Russel (2006) reported that Lys was a good substrate to enrich cultures of *F. necrophorum*

obtained from the rumen. The Lys source utilized in the current study was provided in an encapsulated form, which provided a protective barrier against extensive ruminal degradation. Ruminal disappearance of Lys from nylon bags suspended in the rumen was only 3% after a 16 hour-incubation period, which we deem unlikely to have appreciably altered microbial populations in the rumen. Nonetheless, feeding RPL in the current study increased incidence of liver abscesses in finishing cattle, which is a condition mainly caused by *F. necrophorum*. Such observation leads us to speculate that Lys may stimulate growth of *F. necrophorum* in the gut or liver.

Weiser (1966) and Rezac (2014) observed that animals with healthy ruminal epithelium can present liver abscesses, indicating a non-strict correlation between hepatic abscess and rumenitis. Given that the extent of ruminal bypass of RPL used in this study was relatively high, it is conceivable that Lys stimulates proliferation of *F. necrophorum* in the post-ruminal GIT, and if accompanied by "leaky gut" (Horst et al., 2019), could suggest an alternative means for entry of *F. necrophorum* into the portal blood supply. Additionally, Lys supplementation leads to an increase of plasma Lys levels (Hussein et al., 2016), which is catabolized by the hepatic cell. Abundance of Lys in the plasma and in the hepatic tissue could potentially stimulate *F. necrophorum* proliferation in the liver as well.

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TABLES

Table 3.1. Composition of diets (dry matter basis) fed to finishing steers over 112-day study period.

Item*	0RPL	20RPL	40RPL	60RPL
Alfalfa hay	7.00	7.00	7.00	7.00
Sweet Bran®[1]	20.00	20.00	20.00	20.00
Steam-flaked corn	68.87	68.87	68.87	68.87
Vitamin/mineral supplement[a]	1.91	1.91	1.91	1.91
Ractopamine/RPL premix[b]	2.22	2.22	2.22	2.22
Nutrient composition (DM), analyzed				
Crude protein, %	13.11	13.16	13.22	13.27
NE_m, Mcal/kg[c]	0.95	0.95	0.95	0.95
NE_g, Mcal/kg[c]	0.68	0.68	0.67	0.67
Acid detergent fiber, %	8.14	8.15	8.16	8.17
Lys, %[c]	0.15	0.21	0.27	0.34
Ca, %	0.66	0.66	0.66	0.66
P, %	0.38	0.38	0.38	0.38
K, %	0.67	0.67	0.67	0.67
NaCl, %	0.25	0.25	0.25	0.25

*RPL refers to ruminally-protected lysine treatments as 0 (0RPL), 20 (20RPL), 40 (40RPL), or 60 (60RPL) grams/animal daily.

[1]Wet corn gluten feed (Cargill Starches & Sweeteners North America, Minneapolis, MN).

[a]Consisted of limestone, salt, urea, trace mineral premix, vitamin A premix, and vitamin E premix, to provide (total diet DM) 0.15 mg/kg cobalt; 10 mg/kg copper; 0.5 mg/kg iodine; 20 mg/kg manganese; 0.10 mg/kg selenium; 30 mg/kg zinc; 2205 IU/kg vitamin A; 22 IU/kg vitamin E; and 33 mg/kg monensin (Rumensin; Elanco U.S., Greenfield, IN)

[b]Consisted of premixtures containing ractopamine hydrochloride (Optaflexx 45; Elanco U.S.) fed at the rate of 300 mg/animal daily and RPL (Safegain; H.J. Baker & Bro., LLC.) to supply 0, 20, 40, or 60 g/animal daily of ruminally protected lysine.

[c]Calculated based on the Nutrient Requirement of Beef Cattle, 8th Revised Edition.

Table 3.2. Impact of supplementing of ruminally-protected lysine (RPL) in conjunction with ractopamine-HCl (last 42 days on feed) on performance of finishing steers during the 112-day feeding period.

						P-value	
Item†*	0RPL	20RPL	40RPL	60RPL	SEM	Linear	Quad.
Initial BW, kg	545	546	546	545	8.5	0.92	0.59
DMI, kg/day	10.62	10.76	10.67	10.83	0.159	0.45	0.94
ADG, kg	1.93	1.92	1.95	1.95	0.037	0.63	0.91
G:F	0.182	0.179	0.183	0.180	0.0031	0.83	0.90
Final BW, kg	631	632	634	633	8.9	0.64	0.75

†ME means animals not dosed with *Megasphaera elsdenii* and transitioned with a 21-d step-up (-ME) or dosed (+ME) and transitioned to finishing diets over a 10-d period;

RPL refers to ruminally-protected lysine treatments as 0 grams/animal (0RPL), 20 g/animal (20RPL), 40 g/animal (40RPL), or 60 grams/animal (60RPL) daily.

*DMI = dry matter intake; ADG = average daily gain; and G:F = gain to feed ratio as a measurement of feed efficiency.

Table 3.3. Effect of *Megasphaera elsdenii* (ME) administered at initial processing, and step-up regimen, on feedlot performance of finishing steers throughout the 112-day finishing period.

Item†*	-ME	+ME	SEM	RPL x ME	*P*-value
Initial BW, kg	546	545	18.1	0.57	0.80
DMI, kg/day	10.65	10.73	0.252	0.65	0.85
ADG, kg	1.93	1.94	0.060	0.18	0.74
G:F	0.181	0.181	0.0025	0.11	0.81
Final BW, kg	633	631	18.5	0.20	0.79

†Treatment designated as -ME refers to animals that were transitioned with a 21-day step-up and without *Megasphaera elsdenii*. Treatment designation of +ME refers to animals given a single oral drench of *Megasphaera elsdenii* and subsequently transitioned to finishing diets over a 10-day period;

*DMI = dry matter intake; ADG = average daily gain; and G:F = gain to feed ratio as a measurement of feed efficiency.

Table 3.4. Effect of *Megasphaera elsdenii* (ME) administered at initial processing, and step-up regimen, on carcass characteristics of finishing steers.

Item*	-ME	+ME	SEM	RPL x ME	*P*-value
Liver abscess¥, total %	35.6	41.9	3.55	0.67	0.21
A-, %	10.5	12.8	2.35	0.60	0.49
A_0, %	12.1	13.3	2.44	0.33	0.73
A_+, %	13.1	15.9	2.80	0.48	0.43
Marbling score†	463	447	5.8	0.14	0.03
Longissimus muscle area, cm^2	91.79	93.72	0.755	0.61	0.04
Back fat, cm	1.04	1.04	0.038	0.30	1.00
USDA Quality Grade					
Prime, %	1.05	0.54	0.649	0.79	0.58
Choice, %	81.70	72.43	3.690	0.85	0.03
Select, %	16.73	27.03	3.637	0.82	0.02
Sub-select1, %	0.52	0.00	0.375	0.40	0.32
USDA Yield Grade	2.13	2.08	0.100	0.93	0.47
Yield grade 1, %	17.23	20.14	4.321	0.51	0.46
Yield grade 2, %	53.40	53.25	3.886	0.22	0.98
Yield grade 3, %	28.32	25.02	4.837	0.45	0.46
Yield grade 4, %	1.04	1.62	1.204	0.53	0.62

*Treatment designated as -ME refers to animals that were transitioned with a 21-day step-up and without *Megasphaera elsdenii*. Treatment designation of +ME refers to animals given a single oral drench of *Megasphaera elsdenii* (Lactipro NXT; MS Biotec, Wamego, KS) and subsequently transitioned to finishing diets over a 10-day period.

¥Severity determined as described by Brown et al. (1975).

†500 to 599 = Small degree of marbling; 600 to 699 = Modest degree of marbling.

1Carcasses graded as USDA Standard, Commercial, Utility, or Cutter.

Table 3.5. Effect of supplementing ruminally-protected lysine (RPL) during ractopamine-HCl administration period on carcass characteristics of finishing steers.

						P-value	
Item*§	0RPL	20RPL	40RPL	60RPL	SEM	Linear	Quad
Marbling score†	466	451	449	455	7.9	0.29	0.18
LM area, cm^2	93.38	92.66	91.63	93.36	1.014	0.80	0.20
Back fat, cm	1.03	1.04	1.03	1.07	0.044	0.42	0.69
USDA Prime, %	2.1	0.0	0.0	1.1	0.92	0.44	0.08
USDA Choice, %	78.6	76.9	70.8	82.0	4.78	0.84	0.13
USDA Select, %	19.3	23.2	28.1	17.0	4.70	0.92	0.07
USDA Sub-select[1], %	0.0	0.0	1.0	0.0	0.53	0.66	0.33
USDA Yield Grade	2.1	2.1	2.1	2.1	0.11	0.61	0.89
Yield grade 1, %	16.0	23.2	15.6	20.2	5.15	0.74	0.74
Yield grade 2, %	60.7	45.3	60.4	46.8	5.31	0.24	0.85
Yield grade 3, %	22.4	30.5	21.9	31.9	5.76	0.32	0.86
Yield grade 4, %	1.1	1.1	2.1	1.1	1.46	0.87	0.65

*RPL refers to ruminally-protected lysine treatments as 0 grams/animal (0RPL), 20 grams/animal (20RPL), 40 grams/animal (40RPL), or 60 grams/animal (60RPL) daily.

§LM area = Longissimus muscle area; Back fat was measured as 12^{th} rib fat subcutaneous thickness.

†500 to 599 = Small degree of marbling; 600 to 699 = Modest degree of marbling.

[1]Carcasses graded as USDA Standard, Commercial, Utility, or Cutter.

FIGURES

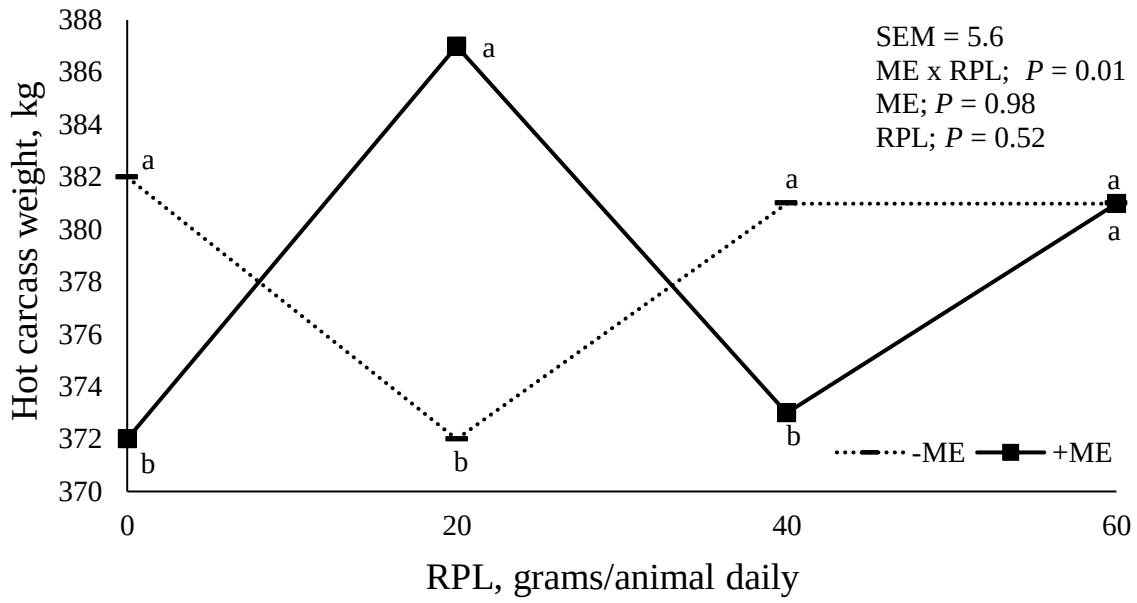

Figure 3.1. Interactive effects of *Megasphaera elsdenii* administered (■ ME) or not (− ME) at initial processing and different amounts of ruminally-protected lysine (RPL) fed during the period of ractopamine administration on hot carcass weight.

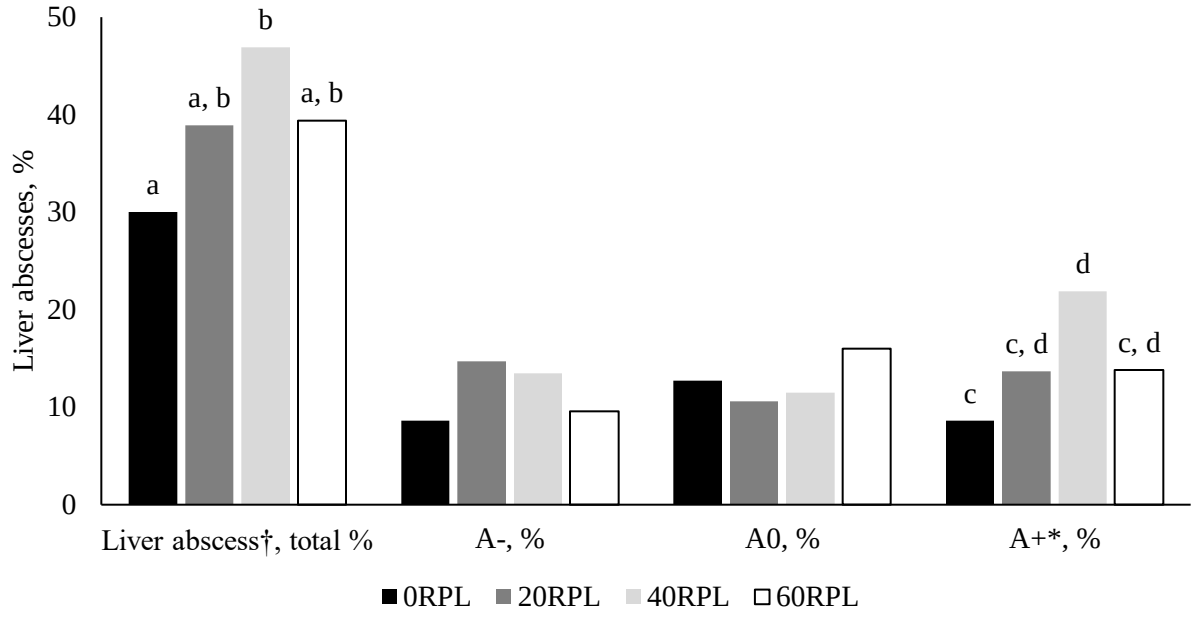

Figure 3.2. Effect of ruminally-protected lysine (RPL) supplementation (0RPL = 0 grams of RPL/animal daily; 20RPL = 20 grams of RPL/animal daily; 40RPL= 40 grams of RPL/animal daily; 60RPL = 60 grams of RPL/animal daily) during ractopamine-HCl administration period on incidence and severity on liver abscesses in finishing steers. Severity was determined as described by Brown et al. (1975).

a, b, c, d Means with a common superscript letter are not different, $P > 0.05$

†Control vs. RPL, $P < 0.05$; Linear effect, $P = 0.11$; Quadratic effect, $P = 0.10$; SEM = 5.02.

*Control vs. RPL, $P < 0.06$; Linear effect, $P = 0.14$; Quadratic effect, $P = 0.07$; SEM = 3.79

Chapter 4 - Effects of ruminally-protected lysine and *Megasphaera elsdenii* on performance and carcass characteristics of finishing cattle

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INTRODUCTION

Understanding amino acid (AA) profile of diets fed to livestock animals is essential to optimize efficiency of protein utilization. Performance of food animals is largely based on their production of proteinaceous products (i.e., beef, milk, eggs). Animal production complements human dietary needs for essential AAs that can be accessed via directly consumption of animal products and their derivatives.

Lysine (Lys) has been identified as the first-limiting AA for cattle fed corn-based diets (Titgemeyer et al., 1988). In cases where microbial protein becomes the primary protein source for ruminants, Lys has been identified as the second-limiting AA (Richardson and Hatfield, 1978). Supplementation of ruminally-protected lysine (RPL) was shown to improve performance of backgrounding heifers fed corn-based feedlot diets (Veloso et al., 2016); however, when RPL was fed in conjunction with β-adrenergic agonists (ractopamine-HCl; BAA), no growth response was observed, but there was an increase in the incidence and severity of liver abscess in feedlot steers (Veloso et al., 2018). *Fusobacterium necrophorum* has been identified as the primary pathogen associated with liver abscesses in beef cattle (Nagaraja &

Chengappa, 1998). Inflammation of the ruminal wall is a predisposing factor that allows bacteria to traverse the ruminal epithelium and subsequently migrate via portal circulation to the liver, where they colonize and induce formation of the abscesses (Nagaraja & Chengappa, 1998). High-grain diets predispose cattle to ruminal acidosis, which can lead to inflammation of the ruminal wall, thus creating conditions that are favorable for pathogen invasion. *Fusobacterium necrophorum* is a proteolytic bacterium, and its proliferation is promoted in Lys enriched medium (Russel, 2006). Lactic acid is a product of ruminal carbohydrate fermentation, and its accumulation in the gut frequently is associated with development of ruminal acidosis. *Megasphaera elsdenii* (ME) is the predominant lactic acid utilizing bacterium in grain-adapted cattle that can be administered as a probiotic to avoid lactate accumulation in the gut. Additionally, ME is an AA fermenting bacterium (Scheifinger et al., 1976) that could additionally compete with *F. necrophorum* for Lys substrate in the rumen. Hence, we hypothesized that administration of ME could decrease incidence and severity of liver abscesses, particularly when promoted by Lys supplementation.

Thus, the objective of this study was to evaluate the impact of ME (Lactipro advance; MS Biotec, Wamego, KS) alone or in combination with RPL (Kemin Industries Inc., Des Moines, IA), on growth performance and carcass characteristics of finishing steers.

MATERIALS AND METHODS

The current study and all procedures used were approved by Kansas State University Institutional Animal Care and Use Committee. The feedlot phase was conducted at the Kansas State University Beef Cattle Research Center in Manhattan, KS, and the laboratory phase was

conducted within the Kansas State University Preharvest Food Safety and Microbiology Laboratory, Manhattan, KS.

Initial Processing and Handling

A lot of 500 crossbred steers were received at the Kansas State University Beef Cattle Research Center in Manhattan, KS. Upon arrival, steers were offered *ad libitum* access to alfalfa hay and water before processing. Within twenty-four hours after arrival, steers were processed through the working facility, where they were individually weighed and uniquely identified with numbered ear tags. Cattle were treated for internal and external parasites using StandGuard (Elanco Animal Health, Indianapolis, IN) and SafeGuard (Merck Animal Health, Summit, NJ), and vaccinated against viral (Bovi-Shield Gold 5, Zoetis Inc., Florham Park, NJ) and clostridial (Ultrabac 7, Zoetis Inc.) pathogens. Cattle observed with signs of illness, injury, poor temperament, or any other abnormalities were excluded from the study, and of the remaining animals a subset of 448 cattle representing the smallest standard deviation for initial body weight (BW) were selected for use in the experiment. After the first assessment of individual weights during initial processing, data were plotted into an Excel spreadsheet and steers were stratified by initial BW, randomly assigned within strata to experimental treatments, and assigned to one of 64 experimental pens with 7 steers each, comprising a total of 16 pens/treatment (i.e., blocks). Study pens provided 5.2 to 7.8 m^2 of space per animal. Pens were partially covered, concrete-surfaced, equipped with automatic waterers between adjacent feed bunks, and provided 0.46 m of bunk space per animal. On day 1 of the study period, individual weights were recorded, steers were implanted with Component® TE-200 (Elanco Animal Health) and then sorted into previously assigned study pens. Eighty-five days after administration of the first implant, steers were re-implanted with Component® TE-200 with Tylan (Elanco Animal Health). Pen weights

were recorded at the end of the experiment, immediately before harvest. Average daily gain (ADG), dry matter intake (DMI), and feed efficiency (G:F) were calculated as indicators of feedlot performance. At the end of the feeding trial, animals were transported for approximately 410 km to a commercial abattoir in Holcomb, KS. The 8 blocks with the heaviest initial BW were harvested after 144 days on feed, and the remaining 8 blocks were harvested after 172 days on feed.

Experimental Treatments

Four hundred forty-eight crossbred steers (352 ± 25 kg initial body weight) were used to determine the impact of ME (MS Biotec LLC.), alone or in combination with RPL (USA Lysine®, Kemin Industries Inc.), on performance and carcass characteristics of finishing steers. Treatments were arranged as a 2 x 2 factorial design with RPL fed at two levels: (1) 0 grams/animal daily (-RPL) or (2) at 40 grams/animal daily (+RPL; 0.45% of diet dry matter); and two step-up regimens: a typical 21-day regimen without ME (-ME), or an accelerated 10-day regimen with ME (+ME; Lactipro NXT; MS Biotec, Wamego, KS). Four intermediate diets were used in both regimens to transition cattle to finishing diets. Freeze-dried ME was re-hydrated and drenched orally on day 1 (1 x 10^{10} CFU/animal) and top-dressed onto diets (dry; 1 x 10^{7} CFU/animal) daily thereafter.

Management and Feeding

Both two step-up regimens used 4 intermediate diets, with concentrate:roughage ratios of: 50:50; 61:39; 72:28; and 82:18 to gradually adapt cattle to the final finishing diet (93:7 concentrate:roughage). For cattle transitioned over the 21-day regimen without ME, each step-up diet was fed for a period of 5 days. For the accelerated 10-day regimen with ME, cattle were fed the step 1 diet for 3 days, and steps 2, 3 and 4 diets for 2 days each. Finishing diets were

composed of (dry matter basis) 60.4% steam-flaked corn, 30% Sweet Bran® (Cargill Corn Milling, Blair, NE), 7% wheat straw, and 2.6% of a vitamin and mineral premix supplement (Table 4.1). The -ME and +ME treatment groups were fed common finishing diets after completion of the step-up regimen. Ractopamine-HCl (Optaflexx 45; Elanco U.S.) was administered as a top-dress at the rate of 400 mg per animal daily during the final 5 weeks on feed. Steam-flaked corn was sampled daily and analyzed for dry matter content (forced-air oven at 105°C for 24 hours) and starch availability. Diets were mixed using a truck-mounted feed mixer (model 490-14; Roto Mix, Dodge City, KS), with 13.9 m^3 capacity. Mixed diets were weighted into plastic barrels and hand delivered once daily to feed bunks, allowing for *ad libitum* access to feed. Unconsumed feed was removed from the bunk as needed, dried at 55ºC for 48 hours, and subtracted from the total amount of delivered feed to allow determination of DMI.

All feed ingredients were sampled weekly, and upon receiving, stored in plastic bags and conserved frozen. At the end of the study period, weekly samples were composited by month and sent to SDK Laboratories (Hutchinson, KS) for analysis of nutrient composition. Lysine content of the premix was analyzed using an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA).

Carcass Characteristics

Animals were harvested on day 144 (32 pens) or day 172 (32 pens) of feeding period. Final shrunk BW was calculated by multiplying pen weight by 0.96 (i.e., 4% pencil shrink). Harvest order, hot carcass weight (HCW), and incidence and severity of liver abscesses were recorded by Kansas State University trained personnel. Incidence and severity of liver abscesses were determined using the procedures described by Brown et al. (1975) where score 0 were attributed to livers with no abscess; A- to livers with one or two small abscesses (measuring 2

cm in diameter or less), or inactive scars; A_0 to livers with one or two large abscesses or several small abscesses; and A+ to livers with multiple large abscesses, greater than 2 cm in diameter, accompanied by inflammation of the organ surrounding the abscess, often affecting collateral tissue. Following 36 hours of refrigeration, carcasses were ribbed and longissimus muscle (LM) area, 12_{th}-rib subcutaneous fat thickness, marbling score, and USDA Yield Grade were determined by camera images. Concomitantly, USDA Quality Grade and incidence and severity of dark cutting beef were determined by a USDA grader. Dressing percentages were determined by averaging HCW for animals within a feedlot pen and dividing that value by final shrunk BW of the pen.

In Situ Determination of Ruminal Digestibility of Ruminally-Protected Lysine

Ruminal stability of RPL was determined via the *in situ* technique and results are illustrated in Figure 4.2 (dry matter disappearance) and Figure 4.3 (crude protein disappearance). Three ruminally-fistulated Jersey steers were used. Animals were housed in a concrete-surfaced pen with *ad libitum* access to water and feed. The basal diet consisted of 45% brome hay, 25.61% steam-flaked corn, 25% Sweet Bran® (Cargill Corn Milling, Blair, NE), and 4.39% of a vitamin-mineral supplement. The *in situ* technique was performed according to standard procedures suggested by Vanzant et al. (1998). *In situ* bags (R510; ANKOM Technology, Macedon, NY) were used to determine ruminal degradability (i.e., dry matter disappearance and crude protein disappearance). One gram of RPL was weighed into the bag (5 cm × 10 cm size and 50 μm porosity) and incubated into the rumen for 0, 8, or 16 hours. Each fistulated animal received two replicates of each of the three treatments (1. SafeGain, H.J. Baker & Bro. LLC., Shelton, CT; 2. USA Lysine, Kemin Industries Inc., Des Moines, IA; or 3. Blank) and incubation time. After removed from the rumen, *in situ* bags were gently hand-washed in buckets where

rinse water was changed until appeared clear. Bags were gently squeezed to remove excess of water and then placed in to a 55° C forced-air oven for at least 48 hours. At the end of the drying cycle, bags were removed from the oven and put into a desiccator to cool down. Residual content was weighed and percent dry matter disappearance (DMD) was estimated as follows:

$$\%DMD = 100*\left(1 - \frac{(\text{Final DM} - \text{Initial Dry Bag Weight})}{(\text{Initial DM})} * \text{Correction Factor}\right)$$

$$\text{Correction Factor} = \frac{\text{Final Blank Bag Dry Weight}}{\text{Initial Blank Bag Dry Weight}}$$

Residual matter was analyzed for protein content using a N analyzer (TruMac™ N Macro Determinator, Leco Corporation, St. Joseph, MI). Adjustment for ruminal bacteria contamination in the residual sample was not performed. Nitrogen content of Lys (encapsulated product) was assumed to be 19.16%, thus protein content was estimated using N x 5.22 as conversion factor. Crude protein disappearance (CPD) was estimated as:

$$\%CPD = \frac{100 * ((((\text{InitialDM} * \%\ \text{InitialCP}) - ((\text{InitialDM} * (1 - (\%\ \text{DMD}))) * (\%\ \text{ResidualCP})))}{(\text{InitialDM} * \text{InitialCP})}$$

Statistical Analysis

Feedlot performance data (initial and final shrunk BW, ADG, DMI and G:F), and non-categorical carcass data (i.e., HCW, dressing percentage, LM area, 12th rib subcutaneous fat thickness, marbling score, and calculated overall yield grade) were analyzed using the MIXED procedure of SAS. Categorical carcass data (i.e., incidence and severity of liver abscess, USDA Quality Grade, and USDA Yield Grade) were analyzed using the GLIMMIX procedure of SAS. Statistical models included fixed effects of RPL, and step-up regimen (i.e., with or without ME),

and the interaction; block was the random effect, and feedlot pen was the experimental unit. Treatment means were calculated using the LSMEANS option, and differences among means were compared using the PDIFF function of SAS. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P < 0.10$.

In situ digestibility was analyzed using the MIXED procedure of SAS. Statistical models included fixed effects of treatment (RPL source), incubation time, and their interaction; random effect were animal and replication within animal; and experimental unit was animal. Least-squares mean differences were compared using the PDIFF function of SAS.

RESULTS AND DISCUSSION

No interactions ($P > 0.10$) between RPL supplementation and ME were observed for feedlot performance or carcass characteristics. Therefore, only main effects of RPL and ME will be discussed.

Feedlot Performance

Impacts of RPL on performance of feedlot steers are shown in Table 4.2. Initial BW was similar between treatments when starting the trial ($P = 30$). No differences were observed for final BW ($P = 0.48$), ADG ($P = 0.35$), DMI ($P = 0.87$), or G:F ($P = 0.17$) when comparing performance of finishing steers fed control or RPL supplemented diets. Our findings are in agreement with those described by Klemesrud et al. (2000b), who supplemented RPL for a 161-day finishing period. When looking at different time periods in their study, Klemesrud et al. (2000b) described treatment effects on ADG and G:F during the first 56 days on feed by supplementing 3 to 4 grams of RPL daily ($P < 0.05$). Average initial BW of steers used in the Klemesrud et al. (2000b) study was 237 kg, whereas animals in the current study had an average

initial BW of 352 kg. The response observed by Klemesrud et al. (2000b) during the first 56 days on feed was most likely due to greater requirements of young animals. Animals that are more physiologically mature, likely deposit proportionately more adipose tissue compared to younger counterparts, and thus may have lower requirements for AA. Lack of response when supplementing RPL to finishing steers fed corn-based diets ($P > 0.05$) also was reported by Oke et al. (1986) when feeding 368 kg feedlot steers; and Hussein and Berger (1995) when evaluating effect of ruminally-protected lysine and methionine on performance of growing and finishing Holstein steers fed corn-based diets. Thus, growth of larger finishing steers fed corn-based diets does not appear to be limited by Lys concentrations of diets.

Effects of ME and transition regimen on finishing steers are shown in Table 4.3. Again, initial BW was similar between treatments ($P = 0.59$). There were no adverse impacts of using an accelerated transition regimen with ME, as final BW ($P = 0.84$), ADG (P = 0.77), DMI ($P = 0.48$), or G:F ($P = 0.31$) were similar between treatments. Steers dosed with ME and transitioned over a 10-day period consumed approximately 17% less roughage compared to their counterparts transitioned with a traditional 21-day step-up program ($P < 0.01$). Observed decreases in forage consumption throughout feeding period was driven by the accelerated transition regimen (10 day) given to ME dosed groups as illustrated in Figure 4.1. Miller (2013) also reported a 17% decrease in silage consumption over a 115-day feeding period when placing feedlot steers directly onto finishing diets ($P < 0.01$). When Ellerman (2017) studied the impact of ME on ruminal characteristics and feedlot performance of finishing steers, no negative effects were observed when an accelerated step-up regimen was used to transition cattle dosed with ME. Hence, transitioning steers with ME and an accelerated step-up regimen seems to yield similar

performance to that achieved using more traditional step-up regimes, which typically are three or more weeks in duration.

Ruminal Degradability of Ruminally-Protected Lysine Sources

Ruminal degradability of two commercially available RPL products (SafeGain, H.J. Baker & Bro., LLC.; and USA Lysine, Kemin Industries Inc.) were evaluated and results are illustrated in Figure 4.2 (dry matter disappearance; DMD) and Figure 4.3 (crude protein disappearance; CPD). Interactions between treatment and time ($P < 0.01$) were observed for DMD and CPD, whereas each RPL source (i.e., SafeGain or USA Lysine) was affected differently at each time point of ruminal incubation. Ruminal DMD and CPD were less than 5% for SafeGain (H. J. Baker & Bro. Inc.) over the entire 16-hour incubation period, and they were not different among the three incubation time points (i.e., 0, 8, and 16-hour incubation period; $P > 0.1$). Protective coat of SafeGain was estimated to provide a ruminal bypass rate of approximately 97% after a 16-hour ruminal incubation period. Ji et al. (2016) evaluated the effect of mixing on *in situ* Lys release of different RPL sources and reported a 17% ruminal disappearance for control MetaboLys treatment (H.J. Baker & Bro. LLC.), and a 95% ruminal degradation for control USA Lysine treatment (Kemin Industries Inc.), after a 12-hour ruminal incubation period. In the current study, USA Lysine had an approximately ruminal DMD of 45%, and ruminal CPD of 66%, over the entire 16-hour ruminal incubation period. Both ruminal DMD and CPD increased as incubation time of USA Lysine increased ($P < 0.05$).

Analysis of RPL sources were performed in samples that were not submitted to mechanical mixing, which was described by Ji et al (2016) to impact ruminal Lys release of RPL products. Thus, our current assessment to ruminal stability of RPL products may be underestimated in comparison to those previously submitted to mechanical mixing.

Carcass Characteristics

Effects of RPL supplementation on carcass characteristics of feedlot steers are shown in Table 4.4. Feeding RPL had no effect on HCW ($P = 0.50$), dressing percentage ($P = 0.95$), LM area ($P = 0.22$), 12th-rib subcutaneous fat thickness ($P = 0.19$), total incidence of liver abscesses ($P = 0.42$), marbling score ($P = 0.19$), or USDA Quality Grades ($P > 0.22$) of feedlot steers. Control steers, not supplemented with RPL, tended ($P = 0.09$) to present more moderate liver abscesses than steers supplemented with RPL. Additionally, feedlot steers supplemented with RPL tended ($P = 0.07$) to have greater overall yield grade than steers without. Oke et al. (1986) investigated RPL supplementation in growing and finishing Angus steers fed corn-based diets, and similarly reported no response to RPL. Likewise, Hussein and Berger (1995) reported no advantageous impact of RPL supplementation on carcass characteristics of finishing Holstein steers ($P > 0.10$). When Hosford et al. (2015) supplemented RPL to finishing steers, no differences were observed between steers fed BAA and steers fed BAA + Lys for most carcass characteristics ($P > 0.10$); but 12th-rib subcutaneous fat thickness ($P = 0.02$) and calculated yield grade ($P = 0.03$) increased in response to Lys supplementation, which is consistent with the tendency for greater yield grade observed in the present experiment. Klemesrud et al. (2000b) reported no differences in carcass traits of finishing steer calves supplemented with various incremental levels of RPL. Veloso et al. (2018) observed that supplementing RPL in conjunction with ractopamine-HCl increased incidence and severity of liver abscesses in finishing steers; however, such observation was not repeated in the current trial. Differences in response to RPL supplementation may be explained by differences in RPL source (SafeGain vs USA Lysine), and potential differences in the extent to which these products escape ruminal digestion and resulting availability for absorption in the post-ruminal gastrointestinal tract. Ruminal degradation of RPL

sources used in the current experiment, and by Veloso et al. (2016) and Veloso et al. (2018), were determined via *in situ* technique, and results were previously discussed.

Effects of ME supplementation in finishing steers are shown in Table 4.5. There were no differences in HCW (P =0.90), dressing percentage (P = 1.00), LM area (P = 0.90), 12th-rib subcutaneous fat thickness (P = 0.26), liver abscess incidence (P = 0.20) or severity of liver abscesses (P > 0.25) between control (-ME) and animals dosed with ME and transitioned to finishing diets with accelerated step-up period (10 days); however, study animals dosed with ME and adapted to finishing diets over a 10-day step-up period tended to have a greater percentage of USDA Prime graded carcasses (0.5 vs 2.7%; P = 0.06). Miller (2013) reported a tendency for animals dosed with ME and transitioned to finishing diets with an accelerated step-up regimen to have a greater percentage of animals graded Choice when compared to Control groups, transitioned conventionally (P = 0.07). Additionally, steers dosed with ME and transitioned to finishing diets over a 10-day period tended to have a greater percentage of USDA Yield Grade 4 (P = 0.09), and fewer USDA Yield Grade 1 (P = 0.07) carcasses than steers transitioned to finishing diets over a 21-day period. This may be explained by differences in dietary energy density during the differing step-up periods.

IMPLICATIONS

Feedlot performance and carcass characteristics of steers adapted with ME, and adapted to finishing diets using an accelerated step-up program, were mostly similar to that presented by conventionally-adapted cattle; although, steers dosed with ME and rapidly adapted to finishing diets consumed about 17% less roughage over the entire finishing period in comparison to their counterparts adapted using a more conventional, 21-day step-up regimen. Supplementing RPL

to finishing steers fed corn-based diets did not affect feedlot performance or carcass characteristics.

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TABLES

Table 4.1. Composition of finishing diet (dry matter basis) fed to feedlot steers over 144 or 172 days on feed.

Item*	
Steam-flaked corn	60.40
Sweet Bran®	30.00
Wheat straw	7.00
Vitamin/mineral supplement[a]	2.60
Nutrient composition (DM), analyzed	
Crude protein, %	13.71
Neutral detergent fiber	21.41
Acid detergent fiber, %	9.05
Lys[b], %[c]	0.45
Ca, %	0.76
P, %	0.44
K,%	1.24
NaCl, %	0.25

*Optaflexx (Elanco U.S., Greenfield, IN) was premixed with ground corn, to serve as a carrier, and top-dressed onto feed to deliver 400 mg/animal daily during the last 5 weeks on feed.

[a]Consisted of limestone, salt, urea, trace mineral premix, vitamin A premix, and vitamin E premix, and provided (total diet DM) 0.15 mg/kg cobalt; 10 mg/kg copper; 0.5 mg/kg iodine; 20 mg/kg manganese; 0.10 mg/kg selenium; 30 mg/kg zinc; 2205 IU/kg vitamin A; 22 IU/kg vitamin E; and 33 mg/kg monensin (Rumensin; Elanco Animal Health, Greenfield, IN). Ruminally-protected lysine (USA Lysine; Kemin Industries Inc., Des Moines, IA) was incorporated into the vitamin and mineral supplement to provide 0, or 40 g/animal daily.

[b]Calculated based on the Nutrient Requirement of Beef Cattle, 8th Revised Edition

Table 4.2. Effect of ruminally-protected lysine (RPL) supplementation on performance of finishing steers (144- or 172-days feeding period).

Item	-RPL	+RPL	SEM	RPL x ME	*P*-value
Initial BW, kg	352	351	6.3	0.38	0.30
Final BW, kg	667	670	6.3	0.99	0.48
DMI, kg/day	10.06	10.05	0.091	0.83	0.87
ADG, kg	2.01	2.03	0.038	0.79	0.35
Gain:feed	0.1993	0.2017	0.00257	0.89	0.17

Table 4.3. Effect of oral dose of *Megasphaera elsdenii* (ME) and step up regimen on performance of finishing steers (144- or 172-days feeding period).

Item	-ME	+ME	SEM	RPL x ME	*P*-value
Initial BW, kg	352	351	6.3	0.38	0.59
Final BW, kg	668	668	6.3	0.99	0.84
DMI, kg/day	10.08	10.02	0.091	0.83	0.48
ADG, kg	2.01	2.02	0.041	0.79	0.77
Gain:feed	0.1996	0.2013	0.00257	0.89	0.31

Table 4.4. Impact of ruminally-protected lysine (RPL) on carcass characteristics of finishing steers.

Item*	-RPL	+RPL	SEM	RPL x ME	*P*-value
HCW, kg	402	404	3.6	0.32	0.50
Dressing percentage	62.77	62.79	0.199	0.18	0.95
LM area, cm^2	95.33	94.15	0.846	0.19	0.22
12^{th} rib fat thickness, cm	1.38	1.43	0.014	0.76	0.19
Liver abscess[1], total %	17.37	14.55	2.473	0.17	0.42
A^-, %	11.43	11.37	2.151	0.21	0.98
A^0, %	3.18	0.92	1.102	0.30	0.09
A^+, %	2.74	2.72	1.105	0.98	0.99
Marbling score†	460	470	5.56	0.66	0.19
USDA Prime, %	0.91	2.29	0.845	0.69	0.25
USDA Choice, %	81.31	84.09	3.031	0.40	0.44
USDA Select, %	17.32	13.15	2.901	0.64	0.22
USDA sub-Select[2], %	0.46	0.46	0.455	0.16	1.00
USDA Yield Grade	2.45	2.58	0.620	0.89	0.07
Yield Grade 1, %	6.82	5.45	1.963	0.29	0.54
Yield Grade 2, %	46.57	42.27	3.559	0.46	0.36
Yield Grade 3, %	40.66	42.03	3.333	0.79	0.77
Yield Grade 4, %	5.49	9.59	1.962	0.81	0.10

†400 to 499 = Small degree of marbling.

[1]Severity was evaluated as described by Brown et al (1075)

[2]Carcasses graded as USDA Standard, Commercial, Utility, or Cutter

*RPL refers to 40 g/animal daily of ruminally-protected lysine supplementation (+RPL) or 0 g/animal daily (-RPL).

Table 4.5. Carcass characteristics of finishing steers in response to different step-up regimens. Regimens included a 21-day step-up period without *Megasphaera elsdenii* (-ME), or a 10-day accelerated regimen accompanied by oral dosing of ME (+ME).

Item*	-ME	+ME	SEM	RPL x ME	*P*-value
HCW, kg	403	403	3.598	0.32	0.90
Dressing percentage	62.78	62.78	0.199	0.18	1.00
LM area, cm^2	94.68	94.80	0.845	0.19	0.90
12^{th} rib fat thickness, cm	1.38	1.43	0.036	0.76	0.26
Liver abscess[1], total %	18.22	13.70	2.468	0.17	0.20
A^-, %	12.75	10.04	2.151	0.21	0.38
A^0, %	1.82	2.28	1.102	0.30	0.73
A^+, %	3.64	1.83	1.105	0.98	0.25
Marbling score†	466	464	5.54	0.66	0.80
USDA Prime, %	0.45	2.74	0.845	0.69	0.06
USDA Choice, %	84.52	80.88	3.036	0.40	0.31
USDA Select, %	14.56	15.90	2.905	0.64	0.69
USDA sub-Select, %	0.46	0.46	0.456	0.16	1.00
USDA Yield Grade	2.48	2.54	0.062	0.89	0.38
Yield Grade 1, %	8.18	4.09	1.963	0.29	0.07
Yield Grade 2, %	41.82	47.02	3.559	0.46	0.27
Yield Grade 3, %	44.09	38.60	3.333	0.79	0.24
Yield Grade 4, %	5.45	9.63	1.961	0.81	0.09

†400 to 499 = Small degree of marbling.

[1]Severity measured as described by Brown et al. (1975)

*ME refers to animals that receive or not 20 ml dose of *Megasphaera elsdenii* on day 1 (1×10^{10} CFU/animal) and top-dressed dried on feed, daily thereafter to deliver 1×10^7 CFU/animal.

FIGURES

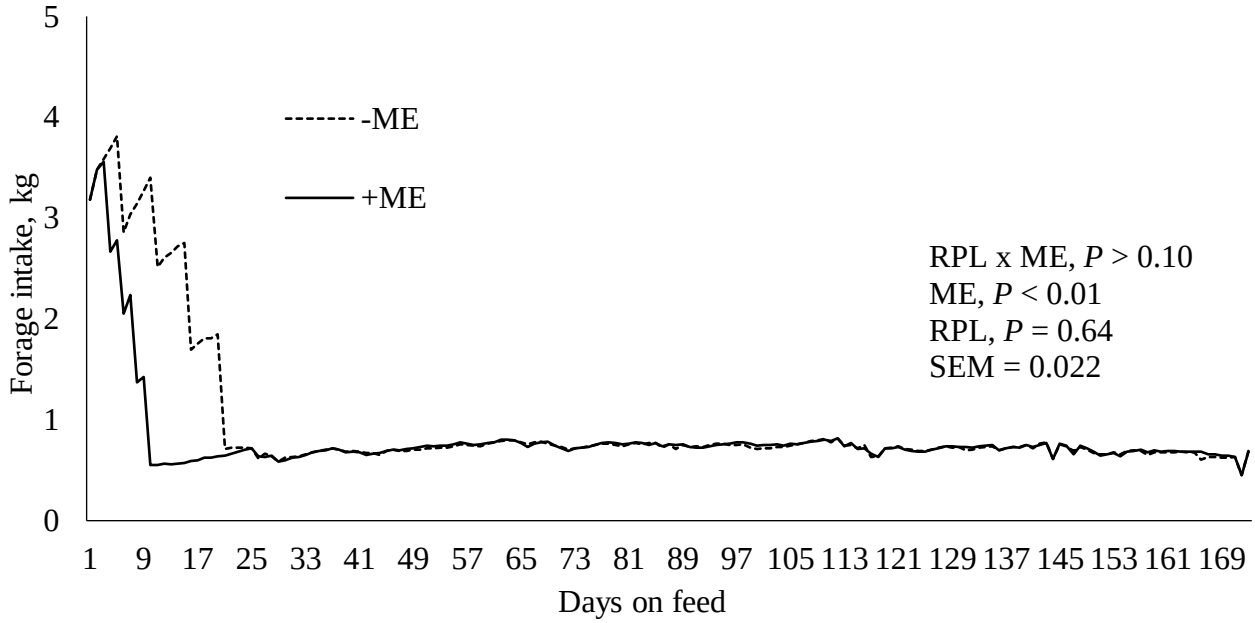

Figure 4.1. Effect of step-up regimen on forage intake of feedlot steers. Regimens consisted of a 21-day step-up period without *Megasphaera elsdenii* (-ME), or a 10-day accelerated regimen accompanied by oral dosing of ME (+ME).

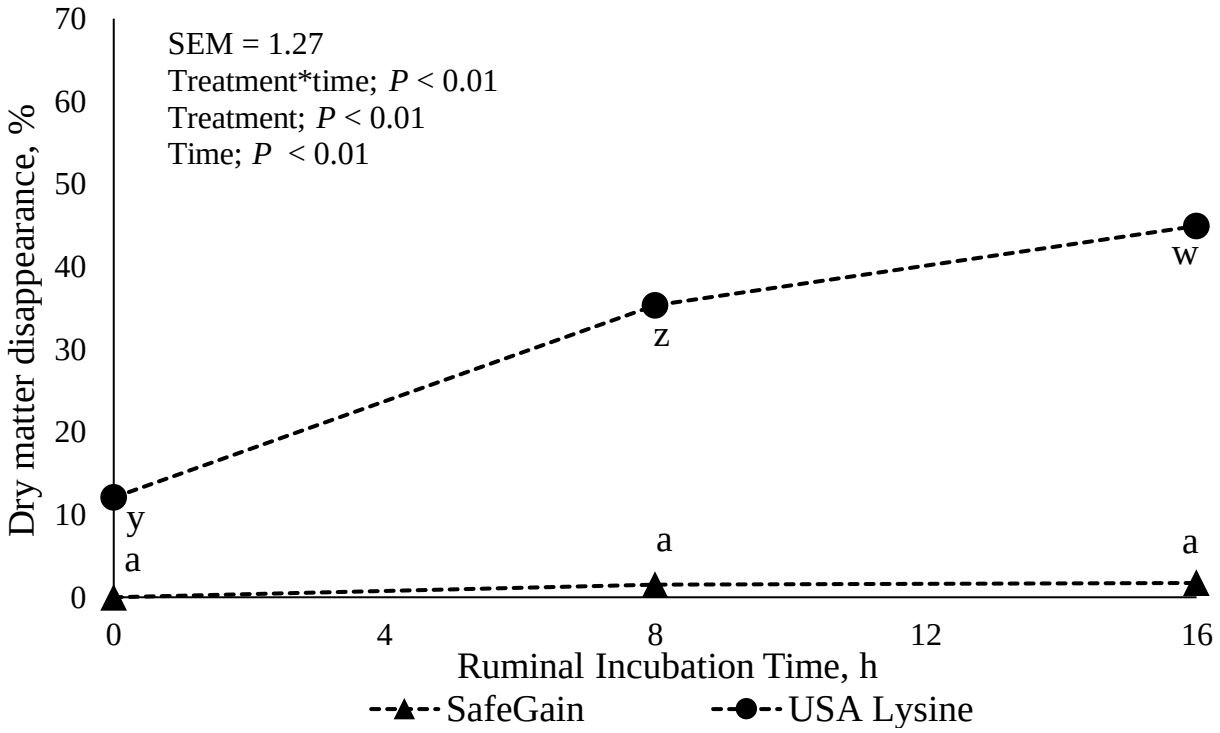

Figure 4.2. *In situ* determination of ruminal dry matter disappearance (DMD) of two ruminally-protected lysine sources: SafeGain (-▲-; H.J. Baker & Bro., LLC., Shelton, CT), and USA lysine (-●-; Kemin Industries Inc., Des Moines, IA).

a, y, z, w Means with a common superscript letter are not different, $P > 0.05$.

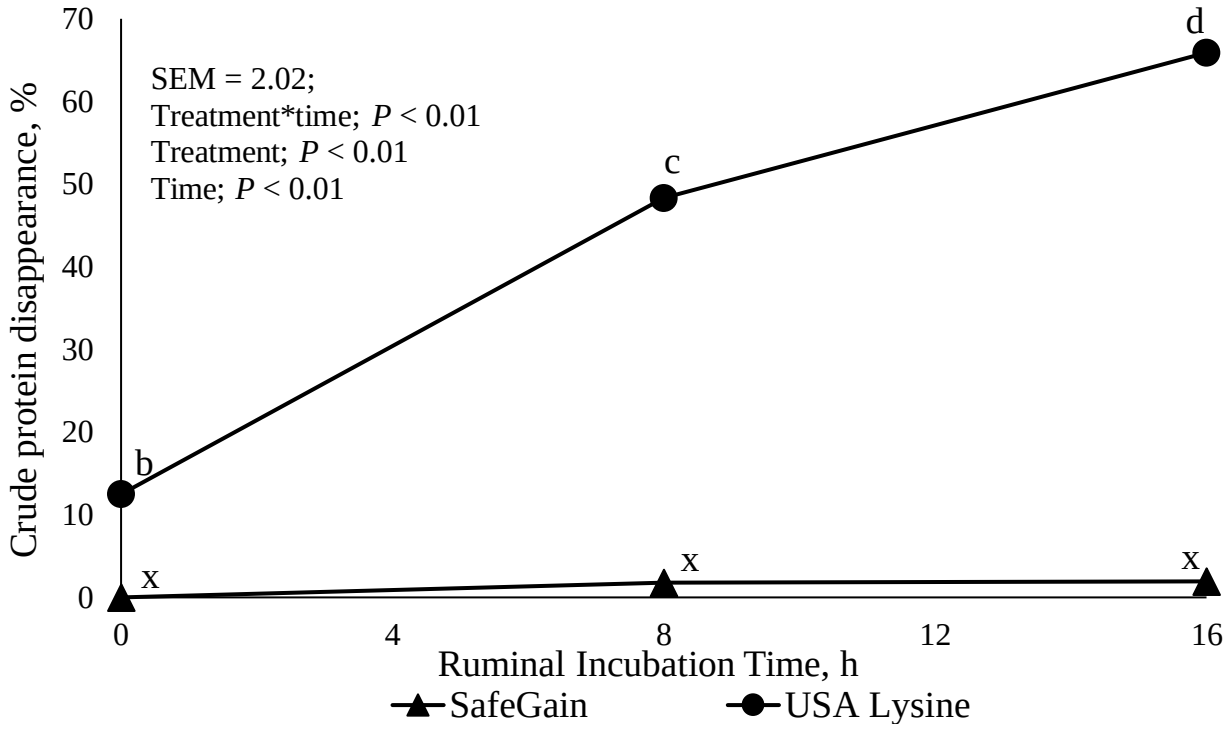

Figure 4.3. *In situ* determination of ruminal crude protein disappearance (CPD) of two ruminally-protected lysine sources: SafeGain (-▲-; H.J. Baker & Bro. LLC., Shelton, CT), and USA lysine (-●-; Kemin Industries Inc., Des Moines, IA).

b, c, d, x Means with a common superscript letter are not different, $P > 0.05$.