The effects of ruminally protected lysine supplementation to growing steers and guanidinoacetic acid supplementation to milk-fed calves.

by

Kathryn Jean Hazlewood

B.S., University of Wyoming, 2020

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2022

Approved by:

Major Professor
Evan C. Titgemeyer

Copyright

Abstract

Studies were conducted to assess growth performance of 1) limit-fed growing cattle when supplemented ruminally protected lysine and 2) milk-fed calves when supplemented guanidinoacetic acid. The first study utilized 340 crossbred steers (250 ± 3.8 kg) in a 77-day growing trial to access the effects of ruminally protected lysine supplementation when limit-fed a corn-based diet. Steers were allocated to 4 blocks with 8 pens, each containing 9 to 12 steers, that were assigned treatments in a randomized block design. Treatments included a control, 3 g /day metabolizable Lys provided by Smartamine ML (Lys-3; Adisseo, Alpharetta, GA), 6 g metabolizable Lys/day from Smartamine ML (Lys-6), and blood meal (BM; AAdvantage; Perdue Agribusiness, Kings Mountain, NC) provided at 0.89% of dietary dry matter to increase metabolizable Lys by 3 g/day plus 2 g/day of metabolizable Met from Smartamine M. Diets included 10% dry-rolled corn, 29.5% steam-flaked corn, 40% wet corn gluten feed, 13% hay, and 7.5% supplement. Steers were limit-fed at 2.4% of body weight once daily. Steers were implanted (40 mg trenbolone acetate, 8 mg estradiol) at trial initiation. Tail vein blood samples were collected on day 14 and 77 for analysis of plasma amino acid concentrations, urea, and haptoglobin. Performance during the growing phase was measured by weekly pen weights. After completion of the 77 days, steers were shipped to a commercial feedlot, mixed into 2 finishing pens, and fed a common diet without treatment for 185 or 206 days then harvested commercially. Performance during the finishing phase was calculated using carcass data acquired from the abattoir. During the growing phase, Lys-3 tended to improve average daily gain by 0.11 kg/day (quadratic, $P = 0.12$) and efficiency of gain (quadratic, $P = 0.08$) compared to control. Plasma concentrations of most amino acids were not affected by supplementation. Plasma urea and haptoglobin were not different between Lys treatments and control ($P > 0.15$). In the finishing

phase, steers previously fed Lys-6 had daily gains 0.05 kg/day numerically greater than control and Lys-3 (P = 0.17). Carcass weights were 7.1 and 3.4 kg heavier than control for Lys-6 and Lys-3, respectively. Bloodmeal did not affect performance during the growing or finishing period. This study indicated ruminally protected Lys supplementation during the growing phase tended to improve performance of cattle during growing and finishing phases and resulting carcass weight. The second study was conducted using 45 (5- to 9-day old) milk-fed Holstein-Angus steer calves (40.9 ± 3.6 kg) supplemented 0, 1, or 2 g guanidinoacetic acid (GAA) per day (Creamino; Alzchem Trostberg GmbH, Trostberg, Germany) in the milk replacer for 42 days. Calves were grouped into blocks of 3 by arrival body weight and total serum protein, and each calf within a block was assigned a different treatment. Daily treatments were divided into 2 daily feedings where calves received 2.84 L of milk replacer (25% protein, 24% fat) at each feeding. Treatment ceased after 42 days and the 17-day weaning period was initiated. From day 42 to 48 calves were fed 1.89 L of milk replacer twice daily, 1.89 L of milk replacer was fed once daily from day 49 to 55, and no milk replacer was provided after day 55. Starter feed and water were provided for *ad libitum* intake for the entirety of the trial. Intake of milk replacer and starter feed were recorded. Health was assessed and scored twice daily for respiratory disease, fecal consistency, and lameness during treatment provision and once daily during the weaning phase. Body weight and hip height were measured on day 0, 14, 28, and 42. Body weight was also measured on day 59 (weaning weight). Due to death loss and removal of 1 calf, performance data of 41 calves were analyzed as a randomized block design. There was a tendency for body weight to linearly increase with increasing GAA by day 59 (linear, P = 0.09). Hip height increased with increasing GAA on day 14 (linear, P = 0.005). With GAA supplementation, there were tendencies for increases in average daily gain from day 0 to 42 (linear, P = 0.15) and day 42 to

59 (linear, $P = 0.14$). Overall, average daily gain tended to increase with increasing GAA (linear, $P = 0.09$). During all time periods, starter feed intake tended to linearly increase ($P \leq 0.07$) with GAA supplementation. The 2 levels of GAA supplementation yielded similar results during the trial. No significant differences among treatments ($P > 0.05$) were observed in health scores during treatment or weaning phases. This study suggests inclusion of GAA in milk replacer tended to increase gain of calves, and this was associated with elevated starter intake.

Key words: cattle, corn, lysine, guanidinoacetic acid, milk-fed.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I could not have accomplished this feat without the help of many, so I want to take the time and name a few here. First and foremost, my advisor, Evan Titgemeyer. I have learned more in the last 2 years then I think I did all 4 years of undergrad. It was a rocky start with Covid making everything more difficult, but I would not be the researcher and scientist if not for your patience and guidance through each step. I appreciate all the time taken answering my questions and having conversations discussing the different aspects of just about everything.

Thank you to my whole committee, Dale, KC, and AJ, I appreciate the various knowledge I have gained from each of you. I believe it's always great to learn different perspectives and each of you have provided me with just that, so thank you.

Next comes the rest of my lab group, Maddie and Jessica. I want to thank you for all the help with projects, guidance on class work, and friendship. I highly enjoyed working with and getting to know each of you. Some of the wild adventures we went on will forever be my favorites! The whole grad office really deserves a mention for being an amazing group of people to spend the last 2 years with.

Of course, I have to say a huge thank you to my family. Moving to Kansas was no small task and I appreciate the support, pep talks, and encouragement each one of you have given me. I loved sharing my little piece of Kansas with you on your visits.

Each of my projects took an enormous amount of work and I couldn't have accomplished each of those without some key people. The stocker unit crew was instrumental in making sure my lysine study went off without a hitch. Bill and Dale, you each taught me a lot on my first study that I have carried with me throughout the rest of my program. A special shoutout to Zach Debord for basically being my feeding crew the last month of the study.

For my second study, the whole undergraduate research class put their all into making sure that study stayed afloat through sickness, weather, and wasps, but especially to my TA's and Reese. I absolutely would not have been able to do it without Hannah, Emily and Reese, so thank you for all the time and effort each of you put into that study. With that, I want to say thank you to Dr. Mike Brouk for giving me a quick tutorial of how to raise housed dairy calves. I appreciate all the knowledge and advice you have shared with me over the course of that study.

Chapter 1 - Review of Lysine Literature

Introduction

Protein supplementation in excess of maintenance requirements allows other processes in the body to occur such as growth, pregnancy, and lactation (NASEM, 2016). An efficient, high rate of growth is key in meeting today's market demands; however, this process is complicated in ruminants due to microbial interference prior to absorption in the small intestine. Overall, providing adequate nitrogen to the microbial population is the primary goal to increase microbial protein synthesis, as the main source of protein in ruminants is of microbial origin. Despite this, microbial interference or degradation in the rumen can lead to a lesser quality protein than that originally provided in terms of requirements of the animal. In addition, protein that does escape degradation (ruminally undegradable protein, RUP) in the rumen may not provide adequate amounts of certain amino acids. Due to this, these amino acids can be limiting in the animal and thus prevent maximum growth from being achieved.

In general, methionine (Met) and lysine (Lys) are the limiting amino acids in cattle due to the deficiency of these amino acids in microbial protein (Richardson and Hatfield, 1978). Supplementation of protein is a beneficial strategy to increase supply of limiting amino acids and therefore enhance growth. However, protein sources vary in amino acid profiles and levels of RUP. Corn and its by-products are used to a great extent in cattle diets, especially during the growing and finishing phases. Corn grain is mainly used due to its high starch percentage that provides energy to diets. When processed, corn by-products can be high in protein, especially RUP. Conversely, corn has a low Lys content, containing 0.25% Lys on a dry matter [DM] basis (NASEM, 2016). By-products such as corn gluten meal have a greater Lys concentration, averaging 1.11% (DM basis; USDA, 2022). Yet, in comparison to solvent extracted soybean

meal, another commonly used protein source that contains an average of 3.07% Lys (DM basis; USDA, 2022), corn gluten meal provides only about 1/3 that amount of Lys. When corn gluten meal was fed, plasma Lys as a percentage of total amino acids was 5.3% as compared to 7.5% when calves were fed groundnut meal (Williams and Smith, 1974). Plasma amino acid concentrations indicate that, in diets containing high percentages of corn, Lys is suggested as the first limiting amino acid followed by sulfur-containing amino acids (Titgemeyer et al., 1988). To maintain and even increase growth rates while using corn and corn-based products in cattle diets, it is imperative to understand the requirements of Lys in cattle and how supplementation of ruminally protected products may allow nutritionists to better meet these needs of growing cattle.

Lysine Requirements and Utilization

To determine the amount of Lys required by growing cattle, various methods have been implemented within experiments. These methods include nitrogen retention, growth performance, and plasma amino acid concentrations as response criterion.

Requirement and Supply Models

First, to understand the suggested requirements discussed below, the current models used for calculation will be examined. The current models presented were developed more recently than some trials discussed in later sections below, but the concepts in the models are still relevant and may have even been influenced by the referenced trials. Metabolizable protein (MP), amino acid requirements, and nitrogen balance are important measures to use in determining the needs of growing cattle.

A significant measure to understand while investigating amino acid and protein requirements is MP supply. This will help to predict how much protein will be available to the animal when fed certain feedstuffs. As presented in NASEM (2016), metabolizable protein of a

feedstuff (MP_j; kg/d) can be calculated using 2 approaches. Level 1 is the empirical level of solution that uses tabular values for the feedstuffs in the diet to calculate supply as follows:

$$MP_j = MPfeed_j + MP_{mtpj}$$

To calculate MP supply of a feedstuff, the tabular value for MP of a feedstuff ($MPfeed_j$; kg/d), is added to the bacterial metabolizable protein derived from ruminal fermentation of a feedstuff (MP_{mtpj}; kg/d). To find $MPfeed_j$, the type of feed and RUP content is needed. In forages, the digestibility of RUP in the small intestine is estimated to be 60%. In concentrates, this estimate increases to 80%. Therefore, if the diet is 100% forage, RUP is multiplied by 0.6 to calculate contributions to MP. When the diet contains less than 100% forage, RUP is multiplied by 0.8. The microbial true protein of a feed (MTP; g/d) is derived from the microbial crude protein (MCP; kg/d) provided by fermentation of the feed, or how much microbial protein synthesis occurs when the animal consumes that feedstuff based on dry matter intake (DMI; kg/d). Microbial true protein is recognized as 80% true protein (20% nucleic acids) with an 80% small intestinal digestibility. This results in $MP_{mtpj} = MCP_j \times 0.8 \times 0.8$. Microbial crude protein is estimated in Level 1 from total digestible nutrient (TDN; % DM) and ether extract (EE; % DM) contents of the feedstuff (NASEM, 2016) using the following equations:

$$MCP_j = \begin{cases} (42.73 + 0.087 \times TDN_j \times DMI_j)/1000, & EE < 3.9\% \\ (53.33 + 0.096 \times FFTDN_j \times DMI_j)/1000, & EE \geq 3.9\% \end{cases}$$

Fat-free total digestible nutrient content of a feedstuff ($FFTDN_j$; % DM) can be calculated by subtracting (2.25 × EE) from TDN concentration.

The second approach to estimating MP supply is the mechanistic level of solution or Level 2 of NASEM (2016). This still uses the concept of digested RUP and true bacterial protein as in Level 1. However, Level 2 uses the protein fractions of a feedstuff determined by rumen degradation *in situ* to calculate MP supply:

$$MP_j = PBID_j \times REPB_j + REBTP_j$$

Protein fraction A is the part of dietary protein that is considered completely degraded due to high solubility in the rumen, whereas fraction B can be partially degradable and will partially escape the rumen. Degradation and escape are dependent on the rate of degradation (kd; h^{-1}) and rate of passage (kp; h^{-1}). Rate of degradation is estimated by *in situ* or by *in vitro* methods of measurement. A measured rate or an assumed rate of 6%/h for can be used for kp. In addition, fractional passage rates can be calculated based on type of particle in the rumen (liquid, concentrate, or forage). The third fraction (C) is considered completely insoluble in the rumen. The C fraction, combined with the insoluble segment of fraction B, make up RUP accordingly:

$$RUP = B\,[kp/(kd + kp)] + C \times 100$$

In many feedstuffs, little or none of fraction C can be absorbed in the small intestine, and therefore fraction C does not contribute to MP. Intestinal digestibility of protein fraction B of a feedstuff ($PBID_j$; %), which ranges from 60 to 80%, is then multiplied by the escape portion of protein fraction B ($REPB_j$; kg/d) that is determined by DMI, kp, and kd of fraction B (measured or estimated at 6.7%/h). The amount of fraction B digested in the intestine is essentially RUP digestibility, therefore, the coefficients of RUP digestibility described above for forages and concentrates could be used for this estimate. The product is added to the ruminal escape bacterial true protein ($REBTP_j$; kg/d) to determine MP of the feedstuff (kg/d).

The animal requirement for MP has 2 factors, metabolizable protein for maintenance (MP_m; g/d) and metabolizable protein for gain (MP_g; g/d; NASEM, 2016). The maintenance requirement is estimated fairly simplistically as it is dependent on metabolic shrunk body weight ($SBW^{0.75}$; kg). Shrunk body weight is typically estimated as 96% of full body weight.

$$MP_m = 3.8 \times SBW^{0.75}$$

Metabolizable protein for gain is calculated by dividing the requirement for net protein for gain (NP_g; g/d) by an efficiency factor (efficiency of use of absorbed protein). Net protein is the protein available from MP that is used for protein deposition. The efficiency factor is influenced by the size of the animal, so the efficiency factor is calculated using an equivalent shrunk body weight (EQSBW; kg). The EQSBW scales shrunk body weight to final mature size to create a proxy for physiological maturity of the cattle for estimating the efficiency factor applied to estimation of protein requirements.

$$\mathrm{MP_g} = \mathrm{NP_g} / \max(0.492, 0.834 - 0.00114 \times \mathrm{EQSBW})$$

As an animal gains weight and matures, efficiency of use of protein for gain decreases linearly. Up to 300 kg of body weight (BW), the efficiency factor is dependent on EQSBW, however, after this BW a constant factor of 0.492 is used. Therefore, to make the efficiency factor equation easier to understand, if the equation (0.834 – 0.00114 × EQSBW) results in a number larger than 0.492, that number is used as the denominator. However, if the resulting number is less than 0.492, then 0.492 is used to divide NP_g. Net protein for gain is determined by shrunk weight gain (SWG; kg/d) and retained energy (RE; Mcal/d).

$$\mathrm{NP_g} = \mathrm{SWG} \times (268 - 29.4 \times \mathrm{RE/SWG})$$

Shrunk weight gain is another term for average daily gain (ADG; kg/d) when calculated using shrunk weights (96%). For RE, all required dietary DM needed to support maintenance, lactation, and pregnancy is subtracted from the total DMI and that number is then multiplied by the dietary net energy available for growth (NE_{ga}; Mcal/kg). This term (NE_{ga}) is derived from dietary supply of metabolizable energy consumed from a feedstuff. Metabolizable energy from a feedstuff (Mcal/d) is estimated to be 0.82 of digestible energy of a feedstuff consumed (Mcal/d) that can be corrected for inclusion of ionophores in the diet. To determine the concentration of

consumed metabolizable energy (Mcal/kg), metabolizable energy is divided by DMI. Digestible energy is based on the apparent TDN of a feedstuff (kg/d) and the assumption 1 kg of TDN is equal to 4.409 Mcal of DE.

A model has been developed to determine individual amino acid (i) requirements for both maintenance (MAA_m; g/d) and growth (MAA_g; g/d; NASEM, 2016).

$$\text{Maintenance: } \text{MAA}_{\text{m}_\text{i}} = \text{TissueAA}_\text{i} \times \text{MP}_\text{m}$$

$$\text{Growth: } \text{MAA}_{\text{g}_\text{i}} = \text{TissueAA}_\text{i} \times \text{NP}_\text{g} / \max(0.492, 0.834 - 0.00114 \times \text{EQSBW})$$

Each equation utilizes an average amino acid content in the tissue (TissueAA) of cattle. This is a tabular value and for this literature review the current relevant value for Lys is 6.4 g Lys/100 g protein. For maintenance, this TissueAA value is multiplied by the MP_m requirement discussed previously. In the latter equation, TissueAA is multiplied by NP_g and then divided by an efficiency factor as described for MP_g.

One measure of effectiveness when supplementing protein or amino acids is nitrogen (N) balance, which is an estimate of tissue protein deposition (and may include milk protein and protein deposition associated with pregnancy). This is the difference between N intake and N output. If positive, the difference is assumed to be N retained in the body and, furthermore, assumed to be used for protein deposition. The closer N retention is to N intake, the more efficiently N is retained in the body for use. As presented in NASEM (2016), N intake is based on the crude protein value of the feedstuff (CP_j) and dry matter intake of that feedstuff (DMI_j):

$$\text{N}_\text{Intake} = \sum_{\text{j}=1}^{\text{n}} \left(\left(\frac{\text{CP}_\text{j}}{6.25 \times 100} \right) \times \text{DMI}_\text{j} \times 1000 \right)$$

Absorbed N is calculated from the supply of MP of a feedstuff described above (MP_j):

$$N_{Absorbed} = \sum_{j=1}^{n} (MP_j \times 1000/6.25)$$

Lastly, N retained can either be predicted as the total net protein for gain (NP_g), pregnancy (NP_p; g/d), and milk protein yield (YProtn; kg/d) divided by 6.25 or, experimentally, N retention is determined with the measured N content in urine and feces and calculating the difference from intake, providing the N balance.

$$N_{Retained} = (NP_g + NP_p + YProtn)/6.25$$

$$N_{Retained} = N_{Intake} - (N_{Urine} + N_{Feces})$$

These models aid researchers in predicting requirements of the animal, allowing nutritionists to better meet the requirements by formulating diets to supply the animal with the needed nutrients. In the studies discussed below, calculation of these nutrients may be different, as the models used could be dissimilar to those described above in NASEM (2016).

Nitrogen Retention

Protein deposition can be determined by N balance, the difference between N input and output. When more N is retained by the body, more protein is deposited by the animal, which presumably results in larger gains and thus more product. Nitrogen retention is a direct measure of animal performance and yields acceptable estimates when measured over short amounts of time (Titgemeyer, 2003).

To give evidence of the limitation of corn protein in regard to Lys, Burris et al. (1976) infused varying levels of Lys (0, 12, 24, 36 g/d) into the abomasum. Steers (258 kg) fed a 65% ground shelled corn diet supplemented with 1.2% urea and consuming 4.4 kg DM/d had an increase in N retention of 7.5 g/d from 0 to 24 g Lys/d with 36 g Lys/d having similar results as 24 g Lys/d. When examining N retention in terms of percentage of N infused, retention peaked at

165% of infused N with 24 g Lys/d and then decreased with 36 g Lys/d to 103% of infused N, meaning more N from the diet was retained when 24 g Lys/d was provided to the steer. In a similar experiment, an abomasal infusion of 0 or 24 g Lys/d with varying levels of Met (0, 4, 8, 12 g/d) was provided to steers (230 kg) fed a 65% ground shelled corn diet with urea supplementation (Hill et al., 1980). The average N retention of steers receiving supplementation of Lys with any addition of Met (~17.29 g N retained/d) was greater than the control (13.88 g N retained/d). The greatest retention of infused N (90.4%) resulted from the treatment group of 24 g Lys/d infused without Met with other treatments having ~30-50% less retention. This experiment provided support to the former trial in that addition of 24 g Lys/d post-ruminally enhanced N retention and presumably protein deposition.

In a study without urea supplementation but lacking RUP in the diet, Titgemeyer and Merchen (1991) gave a base abomasal infusion with all essential amino acids, except Lys. Lysine was added to the base infusion in increasing quantities (0, 8, 16, and 24 g L-Lys/d) to determine the Lys requirement in growing cattle (initially 355 kg). As N intake increased with the increasing N associated with treatments, urinary N also increased, resulting in no change in N retention. Therefore, protein deposition (~38 g retained N/d) was not affected by the added Lys. With no response, the authors determined the requirement to be less than 37.8 g Lys/d, the amount provided by the basal diet.

Batista et al. (2016*)* supplemented Holstein steers (165 kg) with one of five levels of Lys (0, 3, 6, 9, 12, or 15 g L-Lys/d) via abomasal infusion to a metabolizable amino acid-deficient diet. A base infusion was provided to supply all essential amino acids except Lys to make Lys limiting. A linear increase from 21.4 to 30.7 g retained N/d was observed from 0 g Lys/d to the calculated break point at 9 g supplemental Lys/d (total supply = 26.5 g metabolizable Lys/d).

Using the linear range observed in Batista et al. (2016), Hussein et al. (2016) used treatment levels of 0 or 6 g of abomasally infused Lys/d. Urinary N excretion was decreased from 51.9 to 44.3 g/d for the respective treatments and N retention was increased from 24.8 to 33.8 g/d with increasing Lys. With 6 g/d of Lys supplemented to the steers (23.9 g metabolizable Lys/d), greater protein deposition occurred.

Young, early-weaned calves (61-64 kg, 7-11 weeks) were fed a flaked corn and corn gluten meal diet and provided 0.260 g Lys/kg BW with or without Met via the reticular groove; they demonstrated increases in N retention of 3 and 5 g retained N/d, respectively, compared to control cattle supplemented with glutamine (Gln; Abe et al., 1997). Similarly, with calves (8-12 weeks, 66 kg) receiving the same treatments, but with a diet including soybean meal instead of corn gluten meal, had 2 g/d greater retained N with the Lys + Met treatment compared to those only provided supplemental Gln. However, no difference was observed when Met was not provided (Abe et al., 1998). This suggests Met may be the first limiting amino acid in young calves as in older animals (Williams and Smith, 1974; Richardson and Hatfield, 1978). In contrast, if Lys is lacking in the diet, such as with corn gluten meal compared to soybean meal, it may become first limiting and supplementation may counter this deficiency. Calves (139 kg, 17 weeks of age) had similar responses to whole protein (casein) when provided 0.120 g Lys + 0.218 g Gln/kg BW via the reticular groove, in terms of N retained as a percentage of intake and percent absorbed (Abe et al., 1997). In comparison to calves receiving only Gln supplementation, the casein and Lys treatment groups had ~5% greater N retention as a percent of N intake and ~6% greater as a percentage of absorbed N. In contrast, calves greater than 3 months of age (13 and 16 weeks, 116 and 138 kg, respectively) receiving 0.260 g Lys + 0.111 g Met/kg BW had only a tendency for a small increase in N retention compared to calves receiving only Gln

supplementation (Abe et al., 1999). With an increase in Lys supply from 15.9 to 16.7 g Lys/d (calculated from BW) resulting in an increase in N retention in young, Lys-deficient calves, the 15.9 g Lys/d was the suggested requirement.

Mixed results were observed by Boila and Devlin (1972) when providing Lys abomasally to steers (270 kg) fed a diet containing 68% corn plus corn gluten meal and supplying 16.6 g Lys/d. Treatments of 3 and 6 g Lys/d had no effect on urinary and fecal N output or N retention compared to the control. However, when 9 g Lys/d was infused (25.6 g Lys/d total), urinary N was increased ~10% of N intake with an equal decrease in N retention. One may speculate high levels of supplemental Lys could cause adverse reactions when provided to cattle. In contrast, studies described above supplemented far greater amounts of Lys without adverse effects. Additionally, Abe et al. (2001) supplemented calves (150 kg) 16, 32, 48, or 64 g Lys/d via the reticular groove and observed no differences in N retention in Lys groups compared to those on the basal diet that was designed to meet requirements. Total DMI decreased as Lys amount increased resulting in no difference in N intake, and urinary, fecal, absorbed, and retained N were not affected by Lys supplementation up to 64 g Lys/d (Abe et al., 2001). These results were congruent with studies mentioned previously where requirements were met by basal amounts of Lys provided by feed (requirement <37.8 g Lys/d). The only reported adverse effect of large amounts of Lys supplementation was diarrhea occurring within 3 hours of administration of 64 g Lys/d (2.0% of DMI). Lysine excretion in both urine and feces was increased with increasing Lys, therefore demonstrating these large amounts of supplemental Lys are economically unreasonable and a waste of usable nutrients. The authors noted diarrhea was not observed in similar trials involving swine.

Not only is the requirement for an amino acid important to know but the efficiency of use

by the animal is also important. Titgemeyer and Merchen (1991) calculated the utilization of Lys to be not greater than 54%, based on estimates of Lys deposition and Lys supply. Significantly, this calculation does not take into consideration maintenance requirements. Batista et al. (2016) observed an efficiency of utilization of only 40% between 0 to 9 g supplemented Lys/d; however, there was inconsistency between the two calculations. The former utilized 8.64% Lys content in deposited protein (Titgemeyer and Merchen, 1991) versus 6.4% in the latter (Batista et al., 2016), possibly due to varying information available when studies were conducted. When maintenance requirements are taken into consideration, the limitations of using N retention can be observed. Calculated maintenance requirements were 9.9 g Lys/d for steers weighing 165 kg (Batista et al., 2016). The basal diet provided 7.6 g/d of usable Lys for growth (17.5 g total/d - 9.9 g maintenance/d). However, Batista et al. (2016) calculated that 8.6 g/d of Lys was deposited. This results in an efficiency above maintenance being greater than 100%. Nitrogen retention may overestimate protein deposition through losses of N other than in urine and feces, which would cause overestimates of efficiencies. As well, the maintenance requirement for the calves in the study of Batista et al. (2016) may have been overestimated by the NRC (1996) equations.

Other studies have shown alterations in diet can change the utilization of Lys. Steers were found to have numerical increases in efficiencies of Lys utilization when urea was added to a ruminal infusion at 0, 40, and 80 g/d urea (efficiencies were 51%, 59%, and 69%, respectively). The improved efficiencies were hypothesized to be due to reduced Lys catabolism in the liver or small intestine (Hussein et al., 2016).

Based on N retention, the requirement for Lys is likely between 23.5 and 26.5 g total absorbable Lys/d (Burris et al., 1976; Batista et al., 2016; Hussein et al., 2016). For younger,

smaller calves (7-17 weeks, 61-139 kg) the requirement may be approximately 15.9 g Lys/d (Abe et al., 1997). Utilization of supplemental Lys varies among studies, and alterations to the diet may change those efficiencies of utilization.

Growth Performance

In addition to N retention, growth performance is a good indicator of protein deposition. Weight and ADG can be utilized to evaluate the rate of protein accretion in the body. This is a fairly accurate measure because lean tissue proportionally accounts for the majority of animal BW gain (Titgemeyer, 2003).

Wilkerson et al. (1993) compiled data from 11 performance trials, totaling 543 steers, to estimate MP and amino acid requirements. The trials used cattle (average 253 kg) fed high-roughage diets with supplementation of a urea-containing control diet deficient in MP. Protein sources included blood meal, corn gluten meal, soybean meal, dried distiller's grains, wet distiller's grains, and combinations of blood meal and corn gluten meal, blood meal and feather meal, blood meal + corn gluten meal + feather meal, urea and tryptophan, and soybean meal + tryptophan. Using ADG and equations from previous work and the NRC (1985) authors estimated maintenance requirements for MP = 3.8 x $BW^{0.75}$ g/d, where BW = body weight in kg. In growing animals gaining 0.49 kg/d, 305 g MP/kg of live weight gain was predicted to be the requirement. Based on calculations by Goedeken et al. (1990 a,b), when MP requirement was met, the authors were able to determine amino acid requirements as a percentage of MP. The resulting calculations suggested 31.2 g Lys/d, or 8.0% of the MP requirement for maintenance plus gain of 0.49 kg/d (Wilkerson et al., 1993).

The level of energy in the diet may influence the amount of protein required and, therefore, the Lys requirement. Steers (280 kg) were fed a 60% corn-based diet with a net energy

for maintenance (NE_m) content of 2.02 NE_m/kg supplemented incrementally using a ruminally-protected lysine (RP-Lys) supplement (2, 4, 6, 8, or 12 g RP-Lys/d; Ludden and Kerley, 1998). By assessing breakpoints calculated from ADG and metabolizable Lys supply at 2.25- and 3.0-times NE_m, a maintenance requirement of 24.6 g Lys/d or 4.67 g Lys/Mcal NE_m was determined for 280-kg steers. For gain, an additional 16.28 g metabolizable Lys/kg BW gain would be required. Growth was impacted both by energy level and supplemental RP-Lys. Increasing energy level in the diet from 1.5 to 3.0 times NE_m resulted in a 1 kg/d increase in ADG and an improvement in efficiency from 7.43 to 4.96 kg feed/kg gain. Supplementation of RP-Lys up to 8 g/d linearly increased ADG from 0.85 to 1.28 kg/d with a corresponding decrease in feed:gain (F:G) from 8.64 to 5.66 kg feed/kg gain. In a second experiment using intakes of 2.25 and 3.0 times the NE_m requirement with heavier steers (366 kg), an increase in ADG was observed with the greater level of energy; however, there were no significant differences between supplemental Lys treatment groups (Ludden and Kerley, 1998). The authors attributed this to increased BW in the second experiment. Protein deposition (growth) requires energy to occur, but protein provided in the diet is both a source of energy as well as amino acids required for lean tissue synthesis (Ludden and Kerley, 1998). This relationship (protein:energy) must be taken into consideration to maximize growth efficiency. The required protein:energy ratio is only accurate if protein deposition is a linear function of energy intake.

As an animal matures, the rate of gain starts to diminish as the composition of tissue deposition is shifted from mainly protein to mainly fat. Fat is essentially a high-energy storage tissue. Therefore, the ratio of protein:energy in deposition is ever changing as the animal grows, requiring less protein and more energy with increasing BW (NASEM, 2016). It takes a greater amount of energy to increase weight gain as deposition shifts toward fat rather than protein. To

maintain more gain in the form of lean tissue, the protein:energy supply must be balanced. There may be a possible BW threshold to maintain this relationship due to the composition of deposition, but that threshold is unknown in cattle.

In terms of protein utilization and its effect on protein and amino acid requirements, data from Wilkerson et al. (1993) suggested the MP requirement for gain (305 g MP/kg live weight gain) remained constant and the efficiency of use was altered as body size and rate of gain change. Other animal-based factors (genetics, stage of maturity, intake, etc.) or feed-based factors (protein and amino acid supply, degradation rate, energy content, etc.) may influence amino acid utilization (Ludden and Kerley, 1998). Fast growth rates, large body size, high intakes, and other animal factors that result in more protein deposition will theoretically decrease efficiency due to the greater amount of energy needed to continue gain as BW increases. The amount of protein/amino acid and energy supply in the diet can alter protein deposition, thus altering the efficiency of utilization of each of those components. This contrasts the theory of a constant utilization factor with a varying requirement as BW increases over time.

Using the collected performance data, the suggested requirement for Lys is 31.2 to 32.58 g Lys/d for an animal gaining 0.49 kg/d (Wilkerson et al., 1993). This can also be expressed as 8% of the calculated MP requirement (Wilkerson et al., 1993) or 4.67 g Lys/Mcal NE_m + 16.28 g Lys/kg BW gain (Ludden and Kerley, 1998). Utilization may be affected by a multitude of animal- and feed-based factors.

Plasma Concentrations

Plasma amino acid concentrations are a frequently used response criterion to determine if requirements are met by the nutrients provided to the animal. In theory, the plasma concentrations of an amino acid should stay at a low, constant level and remain there when

supplies do not exceed requirements, then increase when the requirement of that amino acid is exceeded by the supply (Bergen, 1979). Unfortunately, there are a few limitations to this method.

Unlike measuring N retention or growth performance, there is no direct measure of lean tissue deposition when plasma concentrations are quantified. The requirement can be estimated, but there is no distinct relationship between that estimate and additional protein accretion in the animal (Titgemeyer, 2003). Limitations of use of plasma amino acids can be shown by discrepancies in results, such as Batista et al. (2016) with a 6 g/d difference in the suggested requirement when looking at N retention versus plasma amino acid concentrations. In addition, other components are at play within the blood. Each amino acid, hormone, or metabolite can alter others making it impossible to determine the exact cause of changes in plasma concentration levels (Titgemeyer, 2003).

Increasing levels of abomasally infused Lys have been shown to increase plasma Lys concentrations in varying weights of calves with and without the addition of supplemental Met (Boila and Devlin, 1972; Williams and Smith, 1974; Fenderson and Bergen, 1975; Burris et al., 1976; Hill et al., 1980; Titgemeyer et al., 1988; Abe et al., 1997; Abe et al., 1998; Batista et al., 2016). However, multiple studies failed to determine a requirement using plasma concentrations due to a lack of 2-phase responses where a breakpoint could be determined (Boila and Devlin, 1972; Williams and Smith, 1974; Fenderson and Bergen, 1975; Titgemeyer et al., 1988).

When provided only urea in the diet as supplemental protein, steers (258 kg) increased plasma Lys concentrations 2 hours post-feeding with 12 g Lys/d infused abomasally (Burris et al., 1976). A decrease was observed with an additional 12 g/d (total 24 g Lys/d); however, this treatment group also had greater N retention. Authors attributed this decrease in plasma concentrations to greater protein synthesis occurring, as concentrations of other amino acids

(glycine, alanine, and isoleucine) concomitantly decreased in the plasma. In a similar study based on the previous, plasma Lys concentrations of steers (230 kg) increased an average of 240% over the control when 24 g Lys/d was provided (Hill et al., 1980). Steers (110 to 160 kg) gaining 0.4 kg/d were believed to need less than 18.8 g Lys/d, based on what was provided in the basal diet, due to only a linear increase in plasma Lys concentrations when Lys was supplemented. This indicated the requirement was met by basal Lys supplies (Williams and Smith, 1974).

Steers with double the size (274 kg) and twice the daily gain (0.73 kg/d) were estimated to require nearly double the Lys, at 31 g/d (Fenderson and Bergen, 1975). Similarly, although at a much lower amount of Lys provided (11.16 g Lys/d), only a linear increase in plasma Lys was observed in steers of the same size (270 kg; Boila and Devlin, 1972). These studies were not able to definitively suggest a requirement. Rather, authors determined the requirement to be less than or equal to the amount provided by the basal diet, due to failure of plasma concentrations to demonstrate a breakpoint. Batista et al. (2016) found a breakpoint of 3.12 g/d supplemental infused Lys in addition to 17.5 g Lys/d provided by the basal diet to 165-kg steers. This requirement of 20.62 g Lys/d was near the findings of Williams and Smith (1974; 18.8 g Lys/d for ~135 kg steers) with a relative increase in animal body size. When feeding a high corn-silage diet, Titgemeyer et al. (1988) found steers (313 kg) abomasally infused with 6, 12, or 18 g Lys/d, with or without 15 g Met/d, had linearly increased plasma Lys concentrations, independent of Met addition. They were likewise unable to determine a requirement. In contrast, heavier steers (383 kg) with similar weight gains, fed a high corn-based diet, and infused with 4, 8, or 12 g/d supplemental Lys were found to have a requirement of 44 g Lys/d (4 g supplemental + 40 g from base diet) when Met was not co-infused (Titgemeyer et al., 1988). When 9 g Met/d was additionally infused, steers required 48 g Lys/d (8 g supplemental + 40 g from base diet). This

trend would suggest a greater requirement for heavier animals that are gaining weight at a faster rate.

In young, early weaned calves (61 to 64 kg) fed flaked corn and corn gluten meal, the addition of Lys (0.26 g Lys/kg BW) via reticular groove increased plasma Lys concentrations after 4 weeks of supplementation compared to those only supplemented with Gln. This was independent of additional Met supplementation (Abe et al., 1997). Multiple studies have been conducted to compare the effect of Lys supplementation via the reticular groove in young calves on plasma Lys concentrations when compared to whole protein, such as casein, or the addition of Gln. Calves (139 kg) 17 weeks old were supplemented with 0.12 g Lys + 0.218 g Gln/kg BW, 0.327 g Gln/kg BW, or 0.450 g casein/kg BW (Abe et al., 1997). The Lys treatment group responded similarly to control (whole protein) with greater plasma Lys concentration than those supplemented with only Gln, when measured prior to feeding. However, 3 hours after feeding, Lys concentration in the Lys treatment group were ~3 times greater than the whole protein treatment group. Similarly, prior to feeding, calves (168 kg) 20 weeks of age had greater plasma Lys with 21.2 g Lys + 10 g Met + 22 g Gln/d (~0.13 g Lys/kg BW) continuously infused into the abomasum compared to casein, 21.2 g Lys + 27 g Gln/d (~0.13g Lys/kg BW), or 47 g Gln/d. Both groups containing Lys had similar responses after feeding that were greater than the casein and Gln only groups (Abe et al., 1997).

In similar calves (8-12 weeks, 66 kg) but fed flaked corn and soybean meal, plasma Lys concentrations were increased when Met (0.111 g/kg BW) was included in supplementation with 0.26 g Lys/kg BW compared to Gln-only supplementation, but no effect was observed with only Lys supplementation (Abe et al., 1998). This suggested Met may be the first limiting amino acid in young calves as in older animals as discussed previously. In contrast, calves (116 kg) older

than 3 months of age provided the same rate of Lys and Met (0.26 g Lys + 0.111 g Met/kg BW) did not have an increase in plasma Lys concentrations until 3 hours after feeding in one trial. Conversely, an increase was observed in calves (138 kg) in a separate trial when compared to Gln supplementation (Abe et al., 1999). At all ages, plasma Lys concentrations 3 hours after feeding were increased by Lys supplementation compared to groups supplemented with Gln. When calculated from BW, 15.9 to 16.7 g Lys/d increased plasma Lys concentrations and N retention responses discussed above; this was the suggested requirement.

Increases or decreases in plasma concentration of other amino acids can help to determine if greater protein deposition is possibly occurring and by inference the limiting amino acid requirement has been met. Increases have been observed in plasma concentrations of glutamate, arginine, alanine, glycine, isoleucine, and histidine in response to increasing Lys infusion into the abomasum (Burris et al., 1976; Batista et al., 2016). Plasma concentrations of leucine, serine, tyrosine, valine, and phenylalanine have decreased in response to increasing Lys infusion into the abomasum (Batista et al., 2016; Hussein et al., 2016). Alpha-aminoadipic acid (Lys catabolism metabolite) was increased with greater Lys supply (Batista et al., 2016; Hussein et al., 2016), but had less of an increase with the addition of urea. Authors suggested this indicated greater efficiency of Lys utilization when ammonia was provided in excess, from urea supplementation, in conjunction with Lys supplementation (Hussein et al., 2016).

Utilizing plasma amino acid concentrations to determine Lys requirements has limitations, as differences in weight, growth rate, age, provision of other limiting amino acids, and protein-sparing compounds (urea) could alter the results. Based on plasma Lys concentrations in the studies discussed, the suggested requirement appeared to be 16 to 48 g Lys/d (Williams and Smith, 1974; Fenderson and Bergen, 1975; Burris et al., 1976; Titgemeyer

et al., 1988; Abe et al., 1997; Abe et al., 1998; Batista et al., 2016). No effect was measured in plasma concentrations when excessive amounts of Lys (64 g/d) were provided (Abe et al., 2001). However, this is a far greater amount than the lower suggested requirements of 16 to 48 g Lys/d and, thus, the plasma concentration should plateau and be maintained.

Requirement and Utilization Conclusions

The Lys requirement for cattle is difficult to determine as shown by the large range of suggested amounts (16.8 – 48 g/d). Examining the different methods of measurement provides even more confusion, as there is no one correlating answer or range. Following N retention studies, the requirement is suggested as somewhere between 23.5 to 26.5 g Lys/d; growth performance measures suggested 31.2 to 32.58 g Lys/d; plasma concentrations have the largest range of 16 to 48 g Lys/d. As mentioned above, N retention and growth performance are more reliable measures, so would likely lead to a more accurate assessment; however, there are many factors such as BW and rate of gain that need to be taken into consideration.

Lysine utilization by cattle is also variable amongst studies. Based on N retention, 40 and 54% are the measured efficiencies but can be altered to as high as 69% with the addition of dietary urea. There is also the question of how animal and other feed-based factors could alter these percentages. Overall, there is still further research needed to determine a more definitive Lys requirement and efficiency of utilization throughout the phases of cattle production.

Ruminally-Protected Lysine Supplementation

Lysine and other amino acids are rapidly degraded in the rumen (Cottle and Velle, 1989). Due to this, when fed to ruminants, Lys must be protected or coated to escape microbial degradation and be absorbed in the small intestine to meet suggested animal requirements. Some protected Lys products also have the addition of Met to aid in stabilization of the product (Oke et

al., 1986) and to help generate a response to RP-Lys (Titgemeyer et al., 1988). Just as in the studies examining Lys requirements, supplementation of Lys even in a protected form has led to varying results. Nitrogen retention, growth performance, and plasma concentrations can be used to determine the efficacy of ruminally protected products and the effects of supplementation to cattle.

Nitrogen Retention

To understand if and where ruminally protected products are digested and absorbed, N balance can be measured. However, the literature is limited in this aspect. Lambs (35 kg) fed a ground corn-based diet had an increase in N retention when RP-Lys was provided in the diet or infused into the abomasum compared to control, similar to non-protected Lys that was infused into the abomasum (Oke et al., 1986). Non-protected Lys fed in the diet had a similar response in N retention as the control. This provides evidence of poor availability of Lys to ruminants, if not protected, due to bacterial degradation in the rumen.

Post-ruminally, protected products become available for absorption. Montano et al. (2019) found supplementation of RP-Lys increased both post-ruminal and total tract N digestion in Holstein steers (143 kg) fed a diet of 53% steam-flaked corn and 25% distillers grains at 2.8 kg/d with little effect on microbial protein or efficiency. Conversely, Wessels and Titgemeyer (1997) found no difference in N retention of steers (254 kg) when supplemented with RP-Lys. Nevertheless, RP-Lys products have been shown to alter N retention in the body by becoming available for absorption directly by the animal that consequently could enhance growth performance.

Growth Performance

Animals used in N balance studies are usually younger and smaller than cattle in later production phases that are in an environment designed for rapid gains (Wright and Loerch, 1988; Hussein and Berger, 1995). As discussed previously, cattle are fed high percentages of corn or corn-based products in the growing and finishing phases. This could lead to deficiencies in Lys resulting in sub-optimal protein deposition and possibly limited growth. In attempt to mitigate the Lys deficiency, ruminally protected forms of Lys can be supplemented and performance parameters such as ADG, DMI, efficiency and others can be measured to determine its effect when added to diets.

In the growing phase, when cattle are lighter in weight, some positive effects have been demonstrated when cattle were supplemented with RP-Lys (Oke et al., 1986; Williams et al., 1999; Klemesrud et al., 2000a; Xue et al., 2011; Montano et al., 2019). Steers with BW from 128 kg (Montano et al., 2019) to 269.5 kg (Williams et al., 1999) have increased gain and efficiency when supplemented various amounts of RP-Lys during a relatively short period of time (58 to 84 d). On average, ADG increased 0.12 kg/d compared to control groups with a range of 0.10 to 0.16 kg/d (Oke et al., 1986; Williams et al., 1999; Klemesrud et al., 2000a; Montano et al., 2019). This was achieved with a range of RP-Lys supplemented starting at 0.9 g metabolizable Lys (mLys)/d (Kelemesrud et al., 2000a) up to 11 g Lys/d (Montano et al., 2019) being the effective dosages.

Efficiency was increased in some trials as well. Oke et al. (1986) observed a 0.5 kg/kg decrease in F:G. Montano et al. (2019) observed a 0.022 kg/kg increase in gain:feed (G:F) compared to control, as well as an increased final BW. Comparatively, Xue et al. (2011) found when growing bulls (372.9 kg) were supplemented with 10 g mLys/d they had an ADG of 1.39

kg/d versus 1.22 kg/d for non-supplemented bulls. Efficiency of gain also increased compared to control with supplementation. The cattle in the study of Xue et al. (2011) were larger than those in other trials and fed for a longer period of time (98 d) but because bulls grow to a much larger mature weight than steers, this can still be considered the growing phase. In sheep (20 kg), RP-Lys supplementation of 0.4% of DM at 890 g DMI/d (3.56 g mLys/d) for 8 weeks increased ADG by approximately 28 g/d compared to the control and a lower dosage of 0.2% RP-Lys of DM (1.78 g mLys/d; Han et al., 1996). Feed efficiency was improved with the lower dosage (0.2% RP-Lys). Decreases in morbidity of up to 18% in long-haul growing bulls and steers (156 kg) has been observed when 5 g mLys/d was provided by 10 g/d of RP-Lys product (Brazle and Stokka, 1994). Some of the differences between trial results could be due to differences in concentration and availability of Lys in the diet as well as degradability and absorption of the various RP-Lys products.

In contradiction, Wright and Loerch, (1988) observed a greater rate of gain in steers (282 kg) fed a diet with soybean meal as the protein source than steers fed a corn/corn silage diet supplemented with any level of RP-Lys or a urea control. Heiderscheit and Hansen (2020) observed 16 kg less BW gain in steers (304 kg) fed a corn-based diet supplemented with RP-Lys at 0.6% of dietary DM (3.5 g mLys/d) than those fed a negative control diet designed to be deficient in Lys during the growing phase (d 0-56). Average daily gain of steers supplemented with RP-Lys was numerically greater than for steers fed a diet designed as a positive control (0.37% Lys dietary DM) but was less than the negative control by approximately 0.5 kg/d from d 28 to 56 of the trial. Efficiency of gain was also decreased by approximately 20% with the addition of RP-Lys. The unusual responses to supplemental RP-Lys may have resulted from the small number of cattle used for the experiment.

Overall, in growing cattle, this evidence indicates improved performance with supplemental RP-Lys. Unfortunately, the dosage of RP-Lys supplementation required to increase gain and efficiency in growing cattle is still unclear.

In the finishing phase, supplementation of RP-Lys to larger, more mature cattle for an extended period of time in most studies has had no significant effect on cattle performance, although there have been possible benefits to carcass traits (Oke et al., 1986; Wright and Loerch, 1988; Healy et al., 1995; Klemesrud et al., 2000b). Using the same animals from the previous growing study, Oke et al. (1986) fed various levels of RP-Lys to steers (368 kg) for 84 d and observed only a tendency for greater gain with the highest level of supplementation (0.07% of diet DM with an intake of 7.2 kg/d; 5 g ruminally undegradable Lys/d) compared to control. Numerically, at the end of the trial there was an increase in hot carcass weight (HCW) from 277 kg in the control group to 294 kg in the highest-level treatment group. Though not statistically significant, this could be an indication of a possible benefit of RP-Lys supplementation in the finishing phase. The limitation with this data is the treatment groups consisted of only 10 steers per group, making it difficult to accurately detect carcass differences. Similarly, Klemesrud et al. (2000b) found no effect of RP-Lys supplementation to steers (237 kg) during a 161-d trial but observed a numerical increase in HCW in those provided 3 or 4 g mLys/d. The 3 and 4 g mLys/d groups finished with 26 and 22 kg more HCW, respectively, than the control group. The same limitation is present in this study as above, with only 5 steers per treatment group. Consistent with growing phase trials, the only response observed by Klemesrud et al. (2000b) was from d 0 to 56 where the steers were still in or close to the growing phase. Supplementation of 3 or 4 g mLys/d resulted in the greatest response with approximately 0.28 kg/d greater ADG and

efficiency of ~11% greater than control. Using breakpoint analysis, the authors calculated the greatest gain of 2.10 kg/d resulted from supplementing 2.56 g mLys/d.

In Wright and Loerch (1988), steers (313 kg) supplemented RP-Lys at 0.06% of diet DM with an intake of 8.8 kg/d (5.28 g ruminally undegradable Lys/d) had similar responses to those supplemented with soybean meal compared to a urea-only protein source. A higher level of 0.10% of diet DM with an intake of 8.6 kg/d (8.6 g ruminally undegradable Lys/d) trended lower than the controls, resulting in reduced ADG. Steers supplemented RP-Lys started the finishing phase (d 56 to 180) with lesser BW (396 vs. 412 kg, RP-Lys vs. control) after RP-Lys supplementation in the growing phase (Heiderscheit and Hansen, 2020). However, no improvements were observed in the finishing phase with continued supplementation (0.44% RP-Lys of dietary DM; 3.25 g mLys/d) including a decreased ADG over the entire 180-d study compared to control. There were no differences in G:F for the finishing period or carcass characteristics.

Healy et al. (1995) studied Holstein steers (156 kg) but provided supplementation for the longest period (318 d). The results were similar to previous studies, as there were no effects of supplementation, with only tendencies for intake to increase with moderate supplementation of 5 and 10 g/d of RP-Lys product providing 2.75 and 5.5 g mLys/d, respectively. In addition, there were decreases in HCW, dressing percentage, and kidney, pelvic, and heart fat as Lys increased. In contrast, Hussein and Berger (1995) fed Holstein steers (183 kg) for 266 d but observed a positive response to RP-Lys supplementation. In the last 98 d, efficiency of gain increased by 12% with 10 g RP-Lys product/d providing 5 g mLys/d, compared to control. Over the total trial, 5 and 10 g RP-Lys product/d (2.5 and 5 g mLys/d, respectively) increased final weight by approximately 28 kg over control and 9 kg over groups provided 15 g RP-Lys/d (7.5 g mLys/d).

In addition, ADG and G:F were marginally improved. Supplementation with RP-Lys may not be useful during the finishing phase of cattle growth, as shown by little response in performance measures; however, some advantages may be observed in carcass traits.

As mentioned in the previous section, there may be a threshold based on BW of the animal that could change the relationship between protein and energy (i.e., protein deposition is no longer a direct linear function of energy intake) and the required ratio of protein:energy decreases (Ludden and Kerley, 1998). Thus, the large BW of feedlot cattle could possibly explain the lack of results from supplementation of RP-Lys.

Varying feed sources could affect the outcome of RP-Lys supplementation. In many feeding operations, corn or corn-based product is the primary feedstuff with corn silage added at a lower percentage to the diet as a roughage source. Veira et al. (1991) observed increases in gain when 9-month-old, large-framed crossbred steers were fed grass silage at 105% of ad libitum intake and 0.5 kg barley daily supplemented with RP-Lys product providing 8.2 g ruminally undegradable Lys and 2.6 g ruminally undegradable Met/d. Supplemented steers had a 16.3% increase in ADG with no differences in intake resulting in greater efficiency of 6.81 kg/kg F:G versus 7.88 kg/kg with control. Supplementation of RP-Lys could prove useful when fed with other feedstuffs such as grass silage and barley.

There are many additions that can be made to the diet or animals themselves within a regular feeding operation that could alter the response to RP-Lys supplementation including use of urea or ionophore antibiotics in the diet and hormonal implants in animals. Many studies have included urea as a supplemental crude protein source (Oke et al., 1986; Wright and Loerch, 1988; Wright and Loerch, 1988; Hussein and Berger, 1995; Williams et al., 1999; Klemesrud et al., 2000a), and the results follow the trends for growing and finishing phases described above

rather than demonstrating any impact of urea inclusion in the diet on response to Lys supplementation. In addition, few researchers have added monensin to the diet in either growing (Williams et al., 1999; Montano et al., 2019) or finishing (Klemesrud et al., 2000b) trials. The rates of gain and efficiency were similar to other trials without this addition. Trials using hormonal implantation in finishing steers (Healy et al., 1995; Klemesrud et al., 2000b) had responses to Lys supplementation that were similar to trials using non-implanted cattle. There were seemingly no differences between trials that implemented these modifications and those that did not.

Outside of a feedlot setting, cattle on pasture have shown no response to supplementation with RP-Lys (Williams et al., 1999). Holstein heifers (257 kg) grazing low-endophyte fescue pasture over 60 d had no difference in ADG when supplemented with a soyhull and cracked-corn-based supplement with RP-Lys product providing 7.29 g mLys + 1.98 g metabolizable Met/d compared to cattle provided only the soyhull and corn supplement. For the last 30 d, RP-Lys cattle had a numerically lower ADG than control cattle. This was a similar response to an additional treatment group fed the base supplement with 99 g/d of blood meal added. The authors mentioned a large percentage of the trial experienced an elevated temperature accompanied by relatively high humidity, resulting in what they referred to as “danger” and “emergency” zones. This could lead to the reduced gains observed in this study. Overall, RP-Lys supplementation has not proven useful to cattle on pasture.

Using growth performance measures to evaluate the effectiveness of RP-Lys supplementation, it is evident that supplementation would be beneficial to light-weight cattle in the growing stage. Unfortunately, the ideal amount of RP-Lys supplementation is unclear.

Supplementation of RP-Lys to finishing cattle or cattle on pasture does not appear to provide much benefit except some numerical increases in carcass weight.

Plasma Concentrations

Not only does direct, abomasal infusion of Lys increase plasma Lys concentrations but dietary inclusion of RP-Lys can also increase plasma Lys concentrations when incorporated in the diet, indicating the Lys requirement most likely has been met (Titgemeyer et al., 1988). Because Met is also integrated into some RP-Lys products, increases in plasma Lys and/or Met have been observed (Oke et al., 1986; Titgemeyer et al., 1988; Wright and Loerch, 1988; Veira et al., 1991; Klemesrud et al., 2000a; Klemesrud et al., 2000b; Xue et al., 2011). Increases in plasma Met were observed when growing steers (247 kg) were provided the highest level of RP-Lys (7.7 g ruminally undegradable Lys/d) during the growing phase (Oke et al., 1986). When continued in the finishing phase with the same steers (368 kg) provided the highest level (5.04 g ruminally undegradable Lys/d), a tendency for greater plasma Lys and Met concentrations was observed. Similarly, in growing cattle (210 kg), Klemesrud et al. (2000a) measured an increase of plasma Lys when steers were provided up to 5 g mLys/d followed by a smaller response with any greater dosage. According to a break point analysis, maximum gain was achieved at 1 g mLys/d. Additionally, Wright and Loerch (1988) observed greater plasma Met when finishing steers (313 kg) were provided 7 and 8.6 g ruminally undegradable Lys/d versus control groups provided soybean meal or urea as the protein source but there was no difference in plasma Lys. Plasma isoleucine concentrations were also less than those of the soybean meal-fed control group. When 8.2 g ruminally undegradable Lys/d was supplemented via an RP-Lys product to 9-mo old steers fed grass silage and 0.5 kg/d barley, increases in many plasma amino acids were measured including Lys, Met, arginine, and glutamic acid. These increases were accompanied by

a decrease in plasma histidine concentration (Veira et al., 1991). Heiderscheit and Hansen (2020) observed a numerical increase in plasma Lys concentrations with supplementing RP-Lys product at 0.6% of dietary DM (3.5 g mLys/d) compared with a negative control. Conversely, a positive control treatment, containing 0.37% Lys of total dietary DM using other dietary Lys sources, had greater plasma Lys concentrations than both the RP-Lys and a negative control group in the growing phase; no effect was observed in the finishing phase. In sheep, a quadratic response in plasma concentrations of amino acids was observed with increases of Lys, arginine, leucine, threonine, valine, asparagine, glycine, and serine with RP-Lys supplementation at 0.2% of DM (1.78 g mLys/d) compared to the control diet and 0.4% of DM (3.6 g mLys/d; Han et al., 1996).

Neither Klemesrud et al. (2000b) nor Wessels and Titgemeyer (1997) observed a response to RP-Lys fed to similar-weight cattle (237 and 254 kg, respectively), but in differing circumstances. Steers of Klemesrud et al. (2000b) were fed an all corn-based diet at *ad libitum* intake with RP-Lys provided from 0 to 12 g mLys/d, whereas steers of Wessels and Titgemeyer (1997) were provided varying levels of soybean meal (0, 2, 4% dietary DM) in addition to rolled corn, limit-fed for a gain of 1.1 kg/d, and provided 0 or 5 g RP-Lys product/d providing 0 or 2 g mLys/d, respectively. In each of these situations it is possible Lys was not limiting in the diet or, with the size of the animals, the energy requirements exceeded those for protein. Many reasons could explain the lack of response to RP-Lys supplementation in plasma concentrations, but as stated above, plasma concentrations of amino acids do not provide the most accurate assessment of the changing environment and needs within the body. With this reasoning, the increases of plasma amino acids in response to RP-Lys supplementation must also be observed with caution.

Metabolites besides amino acids can be measured in blood to indicate the state of the body. Changes in plasma urea N can reflect increases or decreases of protein deposition. Xue et

al. (2011) measured a decrease in plasma urea N with supplementation of 5 g mLys/d to growing bulls, as well as a linear decrease of total plasma nonessential amino acids and total amino acids up to 15 g mLys/d. In growing steers, plasma urea N numerically decreased with RP-Lys provided at 0.6% of dietary DM (3.5 g mLys/d) compared to a control diet and significantly decreased compared to a positive control using a by-pass soybean meal product to provide Lys (Heiderscheit and Hansen, 2020). The decreases in plasma urea in response to Lys supplementation likely reflect increased protein deposition.

Plasma amino acid concentrations have shown mixed results from RP-Lys supplementation. There are some results relative to the trends described in the section above with growing cattle having positive increases in plasma Lys but no effect in finishing cattle. The decreases in plasma urea N associated with RP-Lys supplementation are indicative of greater protein deposition.

Ruminally-Protected Lysine Supplementation Conclusions

Supplementation of RP-Lys can provide cattle with Lys directly through absorption in the small intestine to aid in meeting the requirement. Growing cattle are more likely to respond positively to RP-Lys supplementation with increased gains and feed efficiency. No large effect has been observed in cattle in the finishing phase. Providing RP-Lys when feeding other feedstuffs besides corn-based products, such as grass silage, seems to have a positive effect on growth. Other alterations to the diet and animals seem to have no effect on the outcome of RP-Lys supplementation to cattle in the respective stages of life mentioned above.

Conclusion

There is evidence to show Lys is a limiting amino acid in cattle receiving microbial protein as the predominant source of MP (Richardson and Hatfield, 1978). Additionally, when

corn is included in the diet at a high percentage, lysine will most likely be the first limiting amino acid (Titgemeyer et al., 1989). Thus, available Lys needs to be provided in an amount that meets the requirements of cattle to optimize gain and efficiency. However, the exact requirement is still unclear. The efficiency of utilization can alter the amount needed; however, the utilization percentage of Lys by the body is also unclear. One can use the suggested requirements and utilization percentages discussed above as a reference but understand these may under- or overstate what is needed by cattle.

Protein supplementation to meet these requirements in cattle is complicated due to microbial degradation of protein and amino acids in the rumen. Corn and corn-based products provide much of the nutrients cattle need but are lacking in adequate amounts of Lys. Supplementation of RP-Lys is a useful solution in certain circumstances. Lighter, younger cattle in the growing phase appear to benefit from RP-Lys supplementation with increased gains and efficiencies. Heavier, older cattle in the finishing phase do not.

Literature Cited

Abe, M., T. Iriki, and M. Funaba. 1997. Lysine deficiency in postweaned calves fed corn and corn gluten meal diets. J. Anim. Sci. 75:1974–1982. doi:10.2527/1997.7571974x.

Abe, M., T. Iriki, M. Funaba, and S. Onda. 1998. Limiting amino acids for a corn and soybean meal diet in weaned calves less than three months of age. J. Anim. Sci. 76:628–636. doi:10.2527/1998.762628x.

Abe, M., T. Iriki, K. Kaneshige, K. Kuwashima, S. Watanabe, H. Sato, and M. Funaba. 2001. Adverse effects of excess lysine in calves. J. Anim. Sci. 79:1337–1345. doi:10.2527/2001.7951337x.

Abe, M., K. Yamazaki, K. Kasahara, T. Iriki, R. Kuriyama, and M. Funaba. 1999. Absence of limiting amino acids in calves fed a corn and soybean meal diet past three months of age. J. Anim. Sci. 77:769–779. doi:10.2527/1999.773769x.

Batista, E. D., A. H. Hussein, E. Detmann, M. D. Miesner, and E. C. Titgemeyer. 2016. Efficiency of lysine utilization by growing steers. J. Anim. Sci. 94:648–655. doi:10.2527/jas.2015-9716.

Bergen, W. G. 1979. Free amino acids in blood of ruminants -- physiological and nutritional regulation. J. Anim. Sci. 49(6):1577-1589. doi: 10.2527/jas1979.4961577x.

Boila, R. J., and T. J. Devlin. 1972. Effects of lysine infusion per abomasum of steers fed continuously. Can. J. Anim. Sci. 52:681–687. doi:10.4141/cjas72-080.

Brazle, F. K., and G. L. Stokka. 1994. The effect of protected lysine-methionine on gain and health of newly arrived calves. Cattlemen's Day Report. 49–50. doi:10.4148/2378-5977.2056.

Burris, W. R., J. A. Boling, N. W. Bradley, and Young, A. W. 1976. Abomasal lysine infusion in steers fed a urea supplemented diet. J. Anim. Sci. 42:699–705. doi:10.2527/jas1976.423699x.

Cottle, D. J., and W. Velle. 1989. Degradation and outflow of amino acids from the rumen of sheep. Br. J. Nutr. 61:397–408. doi:10.1079/BJN19890126.

Fenderson, C. L., and W. G. Bergen. 1975. An assessment of essential amino acid requirements of growing steers. J. Anim. Sci. 41:1759–1766. doi:10.2527/jas1975.4161759x.

Goedeken, F. K., T. J. Klopfenstein, R. A. Stock, and R. A. Britton. 1990a. Hydrolyzed feather meal as a protein source for growing calves. J. Anim. Sci. 68:2945–2953. doi:10.2527/1990.6892945x.

Goedeken, F. K., T. J. Klopfenstein, R. A. Stock, R. A. Britton, and M. H. Sindt. 1990b. Protein value of feather meal for ruminants as affected by blood additions. J. Anim. Sci. 68:2936–2944. doi:10.2527/1990.6892936x.

Han, K., J. K. Ha, S. S. Lee, Y. G. Ko, and H. S. Lee. 1996. Effect of supplementing rumen-protected lysine on growth performance and plasma amino acid concentrations in sheep. Asian-Australas. J. Anim. Sci. 9:309–313. doi:10.5713/ajas.1996.309.

Healy, B. J., R. T. Brandt, and E. C. Titgemeyer. 1995. Effect of rumen escape amino acids and multiple TBA implants on feedlot performance of lightweight Holstein steer calves. Cattlemen's Day Report. 31–33. doi:10.4148/2378-5977.2020.

Heiderscheit, K. J., and S. L. Hansen. 2020. Effect of rumen-protected lysine on growth performance, carcass characteristics, and plasma amino acid profile in feedlot steers. Transl. Anim. Sci. 4:1–9. doi:10.1093/tas/txaa128.

Hill, G. M., J. A. Boling, and N. W. Bradley. 1980. Postruminal lysine and methionine infusion in steers fed a urea-supplemented diet adequate in sulfur. J. Dairy Sci. 63:1242–1247. doi:0.3168/jds.s0022-0302(80)83075-x.

Hussein, A. H., E. D. Batista, M. D. Miesner, and E. C. Titgemeyer. 2016. Effect of ruminal ammonia supply on lysine utilization by growing steers. J. Anim. Sci. 94:656–664. doi:10.2527/jas.2015-9717.

Hussein, H. S., and L. L. Berger. 1995. Feedlot performance and carcass characteristics of Holstein steers as affected by source of dietary protein and level of ruminally protected lysine and methionine. J. Anim. Sci. 73:3503–3509. doi:10.2527/1995.73123503x.

Klemesrud, M. J., T. J. Klopfenstein, and A. J. Lewis. 2000a. Metabolizable methionine and lysine requirements of growing cattle. J. Anim. Sci. 78:199–206. doi:10.2527/2000.781199x.

Klemesrud, M. J., T. J. Klopfenstein, R. A. Stock, A. J. Lewis, and D. W. Herold. 2000b. Effect of dietary concentration of metabolizable lysine on finishing cattle performance. J. Anim. Sci. 78:1060–1066. doi:10.2527/2000.7841060x.

Ludden, P. A., and M. S. Kerley. 1998. Amino acid and energy interrelationships in growing beef steers: II. Effects of energy intake and metabolizable lysine supply on growth. J. Anim. Sci. 76:3157–3168. doi:10.2527/1998.76123157x.

Montano, M. F., J. O. Chirino, B. C. Latack, J. Salinas-Chavira, and R. A. Zinn. 2019. Influence of supplementation of growing diets enriched with rumen-protected methionine and lysine on feedlot performance and characteristics of digestion in Holstein steer calves. Appl. Anim. Sci. 35:318–324. doi:10.15232/aas.2019-01843.

NASEM. 2016. Nutrient requirements of beef cattle. 8th ed. Natl. Acad. Press, Washington, DC.

Oke, B. O., S. C. Loerch, and L. E. Deetz. 1986. Effects of rumen-protected methionine and lysine on ruminant performance and nutrient metabolism. J. Anim. Sci. 62:1101–1112. doi:10.2527/jas1986.6241101x.

Richardson, C. R., and E. E. Hatfield. 1978. The limiting amino acids in growing cattle. J. Anim. Sci. 46:740–745. doi:10.2527/jas1978.463740x.

Titgemeyer, E. C. 2003. Amino Acid Utilization by Growing and Finishing Ruminants. In: J. P. F. D'Mello, editor. Amino Acids in Animal Nutrition. 2nd ed. CABI Publishing, Cambridge, MA. p. 329–346.

Titgemeyer, E. C., and N. R. Merchen. 1991. Lysine requirement of growing steers. Arch. Anim. Nutr. 41:71–76. doi:10.1080/17450399109424733.

Titgemeyer, E. C., N. R. Merchen, and L. L. Berger. 1988. Estimation of lysine and methionine requirements of growing steers fed corn silage-based or corn-based diets. J. Dairy Sci. 71:421–434. doi:10.3168/jds.S0022-0302(88)79572-7.

Titgemeyer, E. C., N. R. Merchen, and L. L. Berger. 1989. Evaluation of soybean meal, corn gluten meal, blood meal and fish meal as sources of nitrogen and amino acids disappearing from the small intestine of steers. J. Anim. Sci. 67:262–275. doi:10.2527/jas1989.671262x.

USDA. 2022. Feed Composition Database. National Animal Nutrition Program. Available from: https://animalnutrition.org/feed-composition-database. Accessed November 1, 2022.

Veira, D. M., J. R. Seoane, and J. G. Proulx. 1991. Utilization of grass silage by growing cattle: Effect of a supplement containing ruminally protected amino acids. J. Anim. Sci. 69:4703–4709. doi:10.2527/1991.69124703x.

Wessels, R. H., and E. C. Titgemeyer. 1997. Protein requirements of growing steers limit-fed corn-based diets. J. Anim. Sci. 75:3278–3286. doi:10.2527/1997.75123278x.

Wilkerson, V. A., T. J. Klopfenstein, R. A. Britton, R. A. Stock, and P. S. Miller. 1993. Metabolizable protein and amino acid requirements of growing cattle. J. Anim. Sci. 71:2777–2784. doi:10.2527/1993.71102777x.

Williams, A. P., and R. H. Smith. 1974. Concentrations of amino acids and urea in the plasma of the ruminating calf and estimation of the amino acid requirements. Br. J. Nutr. 32:421–433. doi:10.1079/BJN19740094.

Williams, J. E., S. A. Newell, B. W. Hess, and E. Scholljegerdes. 1999. Influence of rumen-protected methionine and lysine on growing cattle fed forage and corn based diets. J. Prod. Agric. 12:696–701. doi:10.2134/jpa1999.0696.

Wright, M. D., and S. C. Loerch. 1988. Effects of rumen-protected amino acids on ruminant nitrogen balance, plasma amino acid concentrations and performance. J. Anim. Sci. 66:2014–2027. doi:10.2527/jas1988.6682014x.

Xue, F., Z. Zhou, L. Ren, and Q. Meng. 2011. Influence of rumen-protected lysine supplementation on growth performance and plasma amino acid concentrations in

growing cattle offered the maize stalk silage/maize grain-based diet. Anim. Feed Sci. Technol. 169:61–67. doi:10.1016/j.anifeedsci.2011.05.011.

Chapter 2 - Review of Guanidinoacetic Acid Literature

Introduction

Creatine has a major role in energy metabolism in the body. When phosphorylated to make phosphocreatine (PCr), it is a high-energy substrate used for fast regeneration of adenosine triphosphate (ATP) during times of high-intensity work. It has been suggested creatine synthesis is high in young, healthy, fast-growing vertebrates under anabolic condition (Wyss and Kaddurah-Daouk, 2000) to meet a larger requirement for growing tissues (Brosnan et al., 2009).

Creatine concentration is greatest in sites of high and fluctuating energy requirements such as skeletal and heart muscle, brain, spermatozoa, and photoreceptor cells of the retina (Wyss and Kaddurah-Daouk, 2000). Guanidinoacetic acid (GAA) is the direct precursor to creatine (Brosnan et al., 2009) and has the possibility to be used as a dietary supplement to increase creatine supply and improve performance in ruminants. This literature review will include the basics of creatine and GAA metabolism with an overview of studies that have compared supplementation of creatine and GAA, experimental work conducted with poultry and swine, and GAA supplementation to ruminants.

General Creatine and GAA Metabolism

Creatine Synthesis

Creatine is synthesized in the body in a 2-step process involving 3 amino acids. First, the amidino group from Arg is transferred to Gly to make Orn and GAA (Wyss and Kaddurah-Daouk, 2000). This reaction is catalyzed by L-arginine:glycine amidinotransferase (AGAT). Next, GAA is methylated at the amidino group by *S*-adenosyl-L-methionine:*N*-guanidinoacetate methyltransferase (GMAT), with the result being creatine.

Arginine can be acquired from the diet or produced endogenously from the urea cycle. This amino acid is sometimes referred to as “conditionally” essential in certain populations, such as young and growing animals, meaning at times when the body cannot synthesize enough the requirement must be met from the diet (Brosnan et al., 2009). Studies in neonatal piglets have shown that creatine synthesis takes as much as 20% of the dietary intake of Arg, but this also suggests a large amount of Arg is synthesized by the body (Brosnan et al., 2009). In that research study, ~12% of dietary Gly was used for creatine synthesis. This indicated large amounts of endogenous Gly production, as the piglets deposited greater than 4 times the Gly received in the milk from the sow in their whole-body protein. Methyl groups come from Met contained in *S*-adenosyl-L-methionine (SAM), resulting in *S*-adenosyl-L-homocysteine (SAH) after methylation of GAA to creatine. Creatine synthesis is a substantial consumer of methyl groups, using up to 40% of all labile methyl groups in the adult human (Brosnan and Brosnan, 2007) and 63 to 77% in young neonatal piglets (Brosnan et al., 2009). Methionine is consumed in the diet or SAH can be hydrolyzed to Hcy and remethylated to Met (with methyl groups from betaine or 5-methyltetrahydrofolate). Approximately 35% of dietary Met was used for creatine synthesis in piglets (Brosnan et al., 2009).

Synthesis of GAA is an inter-organ process that requires substrates and metabolites to be transported in the blood (Brosnan and Brosnan, 2007). Guanidinoacetic acid is formed mainly in the kidney by AGAT which corresponds with the site of Arg biosynthesis (Wyss and Kaddurah-Daouk, 2000). High activity of AGAT has also been found in the pancreas, liver, and rat decidua. Formation of GAA is the rate limiting step in creatine metabolism. This reversible reaction is favorable towards GAA synthesis at a pH of 7.2 to 7.5 and can be modulated by dietary (e.g., fasting, protein-free diets, vitamin E) and hormonal factors. In addition, AGAT is repressed both

in activity and expression by elevated serum creatine concentrations (Wyss and Kaddurah-Daouk, 2000). This results in a decrease in circulating GAA concentrations and, therefore, liver creatine production (Brosnan and Brosnan, 2007). From the kidney, GAA is released into the blood stream and taken up by the liver, the main site of creatine production, where it is methylated by SAM in a reaction catalyzed by GMAT. *S*-Adenosyl-L-methionine:*N*-guanidinoacetate methyltransferase has the greatest activity in the liver with high (Wyss and Kaddurah-Daouk, 2000) and intermediate (Brosnan et al., 2009) activity in the pancreas. This irreversible reaction has an optimum pH of 7.5 and has no feedback inhibition. The catalyst, GMAT, however, is cleaved by trypsin, chymotrypsin, and elastase that causes the inactivation of the enzyme and demonstrates a large decrease in affinity for both SAM and GAA if only 1 substrate is present. If both substrates are present, GMAT is considerably more protected from inactivation by trypsin (Wyss and Kaddurah-Daouk, 2000). In humans with renal problems, both AGAT and GMAT production were decreased, showing the importance of the kidney in this synthesis pathway (Wyss and Kaddurah-Daouk, 2000).

Creatine is released from the liver into the circulatory system for uptake by creatine-containing cells. Creatine uptake into cells occurs via the creatine transporter. This transporter is sodium and chloride dependent and allows movement of creatine against a high concentration gradient (Brosnan and Brosnan, 2007). Hormones that increase the activity of Na/K ATPase, such as insulin, increase the transport of creatine into cells. In opposition, similar to AGAT expression and activity, high concentrations of serum creatine decrease the expression and activity of the creatine transporter. This is evidenced by only small increases in muscle creatine concentrations following a large increase in serum creatine concentrations. Guanidinopropionate

and guanidinobutyrate are inhibitory to creatine transport (Wyss and Kaddurah-Daouk, 2000). The transport of GAA can also be mediated by the creatine transporter (Ostojic et al., 2016).

Creatine Kinase System

The creatine kinase (CK) system is the cyclical reaction of ATP and creatine to adenosine diphosphate (ADP) and PCr, respectively (Wyss and Kaddurah-Daouk, 2000). Creatine kinase catalyzes this reversible reaction. Skeletal and heart muscle are the predominant tissue type with greater than 90% of creatine and PCr found there (Brosnan and Brosnan, 2007). The type of skeletal muscle determines the role of creatine and PCr. In fast-twitch muscles, ATP must be regenerated immediately to accomplish short periods of work at a high intensity (Wyss and Kaddurah-Daouk, 2000). This is accomplished by having a large pool or high concentration of creatine and PCr. In addition, the CK system acts as a buffer to the products of ATP hydrolysis and the ATP:ADP ratio (Brosnan and Brosnan, 2007).

With the availability of a phosphate group on PCr to form ATP, the ADP concentration is kept low. An increase in ADP would inhibit certain ATPases needed to move molecules in and out of the cell. As well, high ADP causes adenine nucleotide loss, decreasing the ability for synthesis and leading to high adenosine monophosphate (AMP) deamination. This high ATP:ADP ratio also decreases production of reactive oxidative species produced by the mitochondria (Brosnan et al., 2009). Utilization of ATP can cause pH to decrease with the release of hydrogen atoms; CK requires hydrogen when the reaction is moving toward ATP, thus aiding in maintaining pH (Brosnan and Brosnan, 2007). In slow-twitch muscles, the CK system acts as an energy transport system moving high energy phosphates from production in mitochondrial sites to areas of high ATP utilization in the cytosol (Brosnan and Brosnan, 2007). This occurs through the activity of mitochondrial CK, a subtype of CK, that readily transfers the

phosphate group on ATP, produced by oxidative phosphorylation, to PCr that then diffuses to high ATP-utilizing cytosolic sites. Once dephosphorylated to creatine, it will diffuse back to the mitochondria. Although these roles seem apparent in the differing tissue types, it has been speculated the CK system may have the ability to adapt to the physiological requirements of the tissue (Wyss and Kaddurah-Daouk, 2000).

Degradation and Depletion

Creatine is degraded to creatinine and excreted at a rate of 1.7% of the creatine pool (creatine + PCr) per day (Crim et al., 1976). This conversion is spontaneous and non-enzymatic in the body (Wyss and Kaddurah-Daouk, 2000). The rate of conversion consists of the almost constant degradation of 1.1% creatine and 2.6% PCr resulting in overall conversion rate of 1.7% of the creatine pool. Creatinine diffuses through the tissues to the blood stream and is excreted by the kidneys in urine. Some bacteria produce creatinases and creatininases that degrade creatine and creatinine, respectively. These enzymes have been determined to be relatively stable over a broad temperature and pH range. Degradation of these compounds would provide a nitrogen source for bacteria, but creatinine degradation products may be toxic to the renal system (Wyss and Kaddurah-Daouk, 2000). In most animals, this would not be of much importance, but may be worth consideration in ruminant animals if dietary creatine supplementation is to be attempted.

When creatine uptake is highly decreased or altogether inhibited, muscle integrity may be altered (Wyss and Wallimann, 1994). A large creatine pool is necessary for the maintenance of muscle integrity in cases of metabolic stress. This is evidenced by greater severe muscle degeneration when thyroid toxicosis is imposed in animals with depleted muscle creatine concentrations. An association has been discovered in some human muscle disorders having high

serum but low muscle concentrations of creatine (Wyss and Kaddurah-Daouk, 2000). However, chronic creatine-depleted muscles can compensate to maintain energy metabolism (Wyss and Wallimann, 1994). Fast-twitch muscle fibers will convert to slow oxidative fibers. This causes a decrease in fiber diameter (decrease in diffusion distance), an increase in aerobic capacity of the fiber, and a decrease in glycolytic potential while increasing in glycogen stores. In addition, a 50% increase in major glucose transporters into the cell has been observed. These alterations result in availability of energy for use by the cell.

Dietary Supplementation

Dietary supplementation of creatine is a major business in humans, worth millions of dollars in the United States (Brosnan and Brosnan, 2007). It may be used to mitigate the issue of depletion discussed previously, but more importantly creatine supplementation has been shown to provide many other positive effects. The small intestine has some capacity for absorption of creatine. Mainly, intake comes from consuming muscle meats and dairy products and is believed to be 80% bioavailable. With a typical, omnivorous western diet, humans will obtain 50% of creatine needed from their diet, and 50% from *de novo* synthesis (Brosnan et al., 2009). In humans, supplementation (via creatine monohydrate; CMH) has been shown to increase muscle performance during high intensity work (Wyss and Kaddurah-Daouk, 2000). This has been attributed to increases in muscle stores of PCr and possibly to accelerating PCr resynthesis during the recovery period.

Uptake by muscles is improved by simultaneous consumption of a large amount of carbohydrates, possibly due to greater insulin that upregulates the creatine transporter. Supplementation is stated to have an effect on carbohydrate metabolism such as increased

glycogen stores in muscle as well as reduced magnitude of excretion of supplemental creatine and GAA (Wyss and Kaddurah-Daouk, 2000; Brosnan and Brosnan, 2007).

In addition to carbohydrates, exercise stimulates the uptake of creatine into muscle tissue (Brosnan and Brosnan, 2007). There is a greater effect of creatine supplementation if PCr concentration is low in muscle prior to supplementation. Other effects of creatine supplementation have been a decrease in concentrations of plasma ammonia and hypoxanthine (adenine degradation), a decrease in muscle and blood lactate concentrations due to less anaerobic glycolysis, and reduced loss of ATP from the muscle during and after exercise (Wyss and Kaddurah-Daouk, 2000). However, no effect of creatine supplementation on muscular ATP concentrations has been observed (Brosnan and Brosnan, 2007).

In humans, an increase in muscle mass has been linked with creatine supplementation. However, it appears this effect is only present when exercise is performed concurrently (Brosnan and Brosnan, 2007). In addition to increased muscle mass, greater water retention occurs the first few days of supplementation. These two effects combined can increase total body weight (Wyss and Kaddurah-Daouk, 2000).

Many positive effects have been observed with creatine supplementation, but some side effects also have been noted. Mild asthma, gastrointestinal problems, muscle cramps and strains, and heat intolerance have all been reported in humans (Wyss and Kaddurah-Daouk, 2000). In addition to these side effects, as stated previously, high creatine concentrations in serum depress AGAT expression and activity resulting in a decrease in GAA production and, therefore, creatine synthesis. Also, high serum creatine downregulates the expression and activity of the creatine transporter into tissues. A possible solution to these issues is to supplement GAA rather than creatine.

Guanidinoacetic acid can be obtained from the same foods as creatine, but only small amounts are present (~10 mg/kg of meat; Ostojic, 2016). Various studies have shown GAA to be advantageous when supplemented. Supplementation of GAA to rats and chickens resulted in significant increases in creatine and ATP in muscle (Ostojic, 2016). The results of GAA supplementation in rats and chickens differ from those of creatine supplementation, where only small increases in muscle creatine were shown, as well as little to no change in ATP. In humans, a daily dose of 2.4 g GAA for 12 weeks was determined to be readily absorbed and available for conversion, resulting in ~36% increase in skeletal muscle creatine (Ostojic, 2016). Ostojic et al. (2016) observed equimolar doses of GAA and creatine (3.0 g GAA/d versus 3.4 g creatine/d) supplemented for 4 weeks in healthy men on usual diet and exercise led to greater muscle and brain (measured by magnetic resonance spectroscopy) creatine concentrations compared to baseline. Creatine did not affect the brain creatine status in 2 out of 3 areas observed. The authors suggest the possibility of preferential uptake of GAA by target tissues, such as those mentioned above, with high requirement of creatine. This indicated the possibility GAA was methylated in extra-hepatic tissue, especially the brain that has been suggested to have its own creatine synthesis system. These studies give evidence of GAA supplementation being a possible solution to the negative effects of creatine supplementation. Rather than AGAT being repressed due to increased creatine by supplementation, GAA would continue to be produced in the kidney. In addition, if GAA is extra-hepatically methylated in tissues, the effect of high creatine concentration in serum on the creatine transporter would not affect creatine increasing in the tissues.

Comparatively, rats supplemented with either 4.0 g CMH/kg feed or 3.6 g GAA/kg feed had a 39% increase in muscle creatine with GAA, but a 46% increase with CMH (Stead et al.,

2001). Creatine slightly increased ATP in muscle of the hindlimb, but no difference was measured between ADP or AMP concentrations. Other effects observed have been improved muscle endurance and strength over a placebo after 6 weeks of supplementation with GAA (Ostojic et al., 2015). Additionally, studies show negligible changes in serum Arg concentrations in response to supplemental GAA in humans (Ostojic, 2016). Guanidinoacetic acid supplementation appears to increase creatine concentrations and have minimal side effects, compared with creatine supplementation, with no signs of toxicity and few gastrointestinal problems.

One negative of GAA supplementation is the possibility of elevated Hcy concentrations in the blood (Stead et al., 2001; Ostojic et al., 2016; Ostojic et al., 2016). With greater amounts of GAA in the body, more SAM is converted to SAH during methylation of GAA. This results in more Hcy, which has been linked to cardiovascular and arteriosclerotic disease in humans (Ostojic, 2016). Rats supplemented with either 4.0 g CMH/kg feed or 3.6 g GAA/kg feed had increased plasma Hcy with GAA supplementation but decreased plasma Hcy concentrations with CMH supplementation, both compared to control (Stead et al., 2001). This result has been shown to be dose dependent (Ostojic et al., 2016). This negative effect of GAA supplementation may not be of the utmost importance when considering supplementation to growing animals in terms of vascular problems, but the biological strain on methyl groups and, therefore, the supply of Met and other substrates for remethylation needs to be considered so as to not create deficiencies in these areas.

GAA Supplementation in Poultry and Swine

Guanidinoacetic acid supplementation has proven beneficial in animals such as poultry and swine. Use in commercial settings has been approved in chickens and swine by the European

Food Safety Authority (EFSA) in the European Union and sole use in poultry by the Food and Drug Administration of the United States (FDA). Many benefits of GAA supplementation can be observed when examining the progression of work and use in poultry and swine.

Poultry

Some of the earliest work with GAA in other animals beside laboratory rats was in poultry. Many abstracts can be found from 2007 to 2009 regarding GAA supplementation in chickens, mainly from the same research group. One experiment examines the effects of GAA when supplemented in a creatine-free diet in comparison to CMH as well as a positive control diet supplemented with fish meal (Ringel et al., 2007). The treatments included a negative control (all-vegetable diet), 3 levels of CMH (0.4, 0.8, and 1.2 g/kg feed), 3 levels of GAA equimolar to the 3 respective CMH treatments (0.3, 0.6, 0.9 g/kg feed), an extra level of GAA at 1.26 g/kg feed, and a positive control of fish meal to provide natural creatine. The authors concluded, due to the low creatine concentration in breast muscle tissue, *de novo* synthesis was not sufficient in the negative control diet. Addition of CMH to this diet improved gain and breast muscle creatine concentrations linearly, further providing evidence of deficiency.

The addition of GAA resulted in a response intermediate to the positive control and lowest treatment level of CMH in terms of gain and feed conversion, but still had greater responses than the negative control. Muscle concentrations of GAA were greatest in negative control and the lowest CMH supplement amount and decreased with increasing CMH or GAA supplementation. Muscle creatine was the inverse, increasing levels of CMH increased muscle creatine concentrations, whereas GAA was intermediate but appeared to be a linear response with increasing GAA. The authors concluded from the concentration levels that GAA was absorbed and converted to creatine, which was incorporated into muscle. (Ringel et al., 2007).

This study demonstrates the potential for improvement in poultry performance with GAA supplementation.

In 2009, the EFSA approved GAA as a safe feed additive in chickens for fattening with limits of 0.6 to 1.2 g/kg feed (EFSA, 2009). Through a series of trials (8) with over 8,000 birds total, a positive effect was observed with the addition of GAA. Mainly, an improvement in feed-to-gain ratio (F:G) was observed with some trials having decreases in feed intake and others having increased gain compared to the control. For carcass characteristics, a common effect was a decrease in abdominal fat of chickens fed GAA compared to control groups, along with an increase in breast weight, decrease in muscle pH 4 hours post-mortem, and an increase in meat brightness. An increase in drip loss was observed in 1 study. Creatine and GAA concentrations were measured in muscle and had similar results to those of Ringel et al. (2007). One study measured ATP levels in the breast muscle and reported increased ATP with increasing GAA supplementation. Tolerance tests were conducted with GAA supplemented up to 6.0 g/kg feed. Depressions in weight gain, intake, and immune system were observed with the highest level of supplementation (6.0 g/kg). Some indications of intolerance were also apparent at 3.0 g/kg (EFSA, 2009). Approval by a governing body provides confidence of the potential benefits of GAA supplementation in poultry in a commercial setting.

Michiels et al. (2012) published one of the first journal articles focusing on the effect of GAA on growth performance, carcass characteristics, meat quality, and energy metabolism in broilers. The study consisted of 1-d old male broilers fed a corn and soybean meal diet as a negative control or supplemented with 0.6 g or 1.2 g GAA/kg feed or fish meal (positive control). The starter, grower, and finisher phases were each 13-d periods. During the starter and grower phase, GAA yielded an intermediate result in average daily gain (ADG) and average

daily feed intake (ADFI) between the positive (high) and negative (low) controls. During the finisher phase, GAA led to a greater G:F than the negative control and was numerically greater than the positive control. In addition, the negative control and GAA had less mortality compared to the positive control (0, 0, and 1.7%, respectively). Overall, GAA produced greater final body weight, ADG, and G:F than the negative control and was not different from natural dietary creatine.

In the breast muscle, GAA concentrations were lesser and creatine concentrations were greater in GAA-treated broilers than in those fed negative and positive control diets. Phosphocreatine to ATP ratio was greatest with 1.2 g GAA/kg but statistically similar to 0.6 g GAA/kg and positive control groups. This could mean greater regeneration of ATP occurred, which could lead to more skeletal muscle growth as well as greater provision of energy for contractile activity of proteins, such as in the heart. Insulin-like growth factor-1 (IGF-1), a possible indicator of muscle growth, was elevated only with 1.2 g GAA/kg. Authors determined a relatively high negative correlation (-0.740) between GAA and creatine concentrations and moderately positive correlations between creatine and creatinine concentration (0.408) and PCr:ATP (0.537) in the breast muscle (Michiels et al., 2012). These correlations could allow others to predict concentrations more accurately without testing for multiple metabolites, but further research is needed to confirm these relationships.

Supplementation of GAA had positive effects on carcass yield and meat quality (Michiels et al., 2012). Breast muscle and lower leg yields were greater with GAA than the negative and positive control, respectively. Also, greater water retention occurred with GAA supplementation as shown by greater press and cooking loss for GAA compared to the negative control, but GAA did not differ from the positive control. This differs from human creatine supplementation. In

humans, greater water retention is common with increased dietary creatine but is not always concurrent with an increase in muscle mass. In addition, GAA treatment groups had a slightly lower pH 24 hours post-mortem in the breast muscle and a lighter but yellower color compared to the negative control. The positive control had a lower temperature at 2-, 3-, and 4-hours post-mortem of ~1-2 °C than GAA groups. There were no differences in total carcass composition in the 3 areas of analysis (moisture, crude protein, or crude fat). Besides the few instances mentioned, both levels of GAA yielded very similar results (Michiels et al., 2012). This study provided a broad view of the similar effect GAA supplementation has on performance, but also on meat quality as compared to a natural creatine source in the diet.

No information on the digestibility of GAA in broilers could be found until work conducted in 2016. Tossenberger et al. (2016) conducted 2 trials to accomplish this task. For both trials, male broilers were fed a corn/soybean meal-based diet free of animal by-products. Treatments included a control of the base diet, 0.6 g, or 6.0 g GAA/kg feed. These treatment levels are interesting as 0.6 g GAA/kg is the minimum dose recommended by the EFSA and 6.0 g GAA/kg is the highest level in the tolerance test discussed above. Day-old chicks were fed for 35 days. There was no difference in weight gain or intake with 0.6 g GAA/kg, but both were depressed with 6.0 g GAA/kg compared to control. Breast muscle and liver GAA concentrations decreased, whereas creatine concentrations increased with increasing level of GAA. As well, muscle creatinine was increased in the 6.0 g GAA/kg group compared to the other treatments. Both plasma creatine and creatinine increased with increasing supplemental GAA, resulting in 10 times the concentrations for the high level of GAA. Elevated plasma Hcy was also observed with 6.0 g GAA/kg, showing possible stress on the remethylation pathway.

In the digestibility study, 24 male Ross 308 broilers (2191 g) were fistulated with a colon cannula, this allowed collection of feces and urine to remain separate, followed by initiation of the trial 7 days later on day 34 of age (Tossenberger et al., 2016). The trial consisted of a 4-day adaption period and a 4-d collection period. Authors determined the main route of excretion for the creatine system was in urine. Numerical increases in fecal and urinary excretion of GAA and creatine were measured with 0.6 g GAA/kg, but major increases were observed with 6.0 g GAA/kg. Urinary excretion of GAA was 3.4, 9.5, and 102.6 mg/kg$^{0.75}$/d for control, 0.6 g GAA/kg, and 6.0 g GAA/kg, respectively. Urinary excretions of creatine and creatinine demonstrated a pattern similar to that of GAA with 1.1, 2.1, and 29.4 mg creatine/kg$^{0.75}$/d and 3.4, 4.8, and 67.0 mg creatinine/kg$^{0.75}$/d, respectively, for the 3 treatments. When the body is oversupplied with creatine (via GAA) these results indicate it will adapt to excrete large amounts so as to not accumulate these products. The calculated apparent digestibility was 99.14 and 98.77% for 0.6 g GAA/kg and 6.0 g GAA/kg, respectively. True digestibility was very similar at 99.40% and at 98.80%, respectively. Examining true availability of GAA, when only GAA excretion is considered, availability is only slightly less with 6.0 g GAA/kg (83.28% vs 71.34%). However, if all 3 metabolites (GAA, creatine, and creatinine) losses are accounted for then 0.6 g GAA/kg had a much higher availability (76.2%) as compared to 6.0 g GAA/kg (45.6%). This indicates GAA is highly digestible independent of dosage, however with high concentrations in the diet the actual availability of the compound will be lowered. Using these calculations, the authors created a factorial approach to estimate GAA and creatine requirements in broilers. With *de novo* synthesis assumed to be 66%, digestibility 99%, and utilization of digested creatine 76.21%, they calculated an optimal dietary level of GAA to be 0.77 and 0.68 g/kg in broilers

weighing 1,000 and 2,050 g, respectively (Tossenberger et al., 2016). These calculated needs fall between the EFSA's allowable feeding levels.

The FDA approved use of GAA for poultry in 2016 (FDA, 2021). The Code of Federal Regulations stipulates use must not exceed 0.12% of the total feed (1.2 g GAA/kg feed) in use as a precursor to creatine or to spare Arg in broiler and turkey feeds. This later point has been discovered in poultry and the extent to which GAA spares Arg has been examined by many studies.

As Arg is a main substrate in the production of GAA and therefore creatine, creatine production can use a lot of Arg that may be needed elsewhere in the body such as for protein synthesis for growth, pathways related to health, and other physiological processes (Portocarero and Braun, 2021). Poultry also require all of their Arg to be provided via the diet, because of an absence of endogenous synthesis accompanied by rapid growth that requires a high rate of protein synthesis, which incorporates large amounts of Arg (Ball et al., 2007). Arginine requirements decrease with age of the broiler, but if GAA could be supplemented in replacement of a certain amount of Arg, then potential performance could be maximized with less dietary Arg provision. Equivalencies range from 0.77:1 to 3:1 (Arg:GAA) across studies as gathered in a review by Portocarero and Braun (2021). The explanation of some factors can help to understand this wide range of values. In studies with high Arg deficiencies, GAA was less effective in alleviating the deficiency and maintaining or improving growth. Therefore, in very deficient animals, the equivalency would be low, whereas mildly deficient animals would have a higher equivalency. Also, the indicator of measure is important to consider. When growth parameters are used, such as gain and feed conversion, the equivalency is low. However, when tissue measures are used such as PCr, the high end of the range is observed. For ease of use, an

equivalency for Arg to GAA of 1:1 (wt:wt) was recommended in broilers (Portocarero and Braun, 2021).

More recently, the research scope of GAA supplementation has widened beyond that of standard growth performance and tissue concentrations. Majdeddin et al. (2020) examined GAA supplementation in heat stressed broilers. One-day old male broilers fed a corn/soybean meal diet adequate in nutrients and amino acids were supplemented 0, 0.6, or 1.2 g GAA/kg feed. After 25 days on feed receiving treatments, cyclical heat stress was induced. Treatments were maintained during the heat stress period through the finisher phase (days 26-39). No differences were observed between treatments in body weight gain. During the grower phase (day 10-25) a linear decrease was observed in ADFI partially accounting for a linear decrease in F:G. In the finisher phase (concurrent heat stress), results similar to the grower phase were observed, with both levels of GAA having similar responses. Panting frequency, number of breaths per minute, was decreased linearly as GAA increased. Groups with GAA had numerically less mortality than the control. Over the total trial compared to control, F:G was decreased with added GAA, with similar results between the 2 levels, and a decrease in panting frequency was observed possibly resulting in a greater survival percentage, especially in the last phase. In addition, an increase in the European Production Efficiency Factor was observed with GAA compared to control. This factor takes into account the length of viability, final body weight in terms of age, and total trial F:G. Similar to other studies, even with the addition of heat stress, body weight was maintained with the decrease in ADFI.

Two types of heat stress were evaluated in the study by Majdeddin et al. (2020). Blood and meat concentrations of GAA and metabolites were assessed in acute and chronic heat stress along with other metabolic indicators. Birds in acute heat stress were subjected to conditions for

1 day and then slaughtered on day 26 after 25 days of receiving treatments described above. For chronic heat stress, birds were in heat stress conditions for 14 days and slaughtered on day 39. In the blood, linear decreases in response to GAA supplementation were measured in thrombocytes, lymphocytes (due to decreases in T cells), and total leukocytes on day 26. Similarly, total leukocytes linearly decreased in chronic heat stress birds with increasing supplemental GAA. Authors proposed these findings could mean GAA is altering the cell-mediated immune response to stress. An increase in corticosterone (cortisol) was observed in the 0.6 g GAA/kg group at day 26, but this was not measured on day 39 suggesting GAA may have no real effect on the stress response in the total body. A linear increase in plasma Arg was observed on day 26 and a quadratic response in Gly with 0.6 g/kg being the highest, providing evidence of Arg sparing and possibly Gly sparing. In the breast muscle, linear increases in response to GAA supplementation were measured in PCr, free creatine, total creatine, and PCr:ATP with most results being statistically different in acute and chronic stress with similar responses between 0.6 and 6.0 g GAA/kg. Interestingly, free muscle creatine concentration was lower on day 39 compared to day 26 (~55 versus ~120 μmol/g dry matter [DM], respectively). This could be due to greater creatine use in the muscle for energy related to stress responses from the chronic heat stress. From this evidence, it can be suggested GAA improved heat tolerance while maintaining or even improving performance and increasing the creatine pool in the body.

Overall, many commonalities can be observed when GAA is supplemented in the diet to chickens. Mainly, decreases in feed intake and/or decreases in F:G are observed in most performance studies. This can be corroborated by Portocarero and Braun (2021) who gathered 42 individual performance studies (~18,500 birds) supplemented with 0.6 – 1.2 g GAA/kg into a comprehensive review. Although not directly observed in the studies presented above, 36 of 42

studies had improvements in gain in response to GAA supplementation with 14 demonstrating improvements greater than 5%. Feed conversion was improved by GAA supplementation in 39 studies with 13 having improvements greater than 5% (Portocarero and Braun, 2021). In the studies discussed above, trends in muscle concentrations of GAA and creatine can be detected; when dietary GAA was provided muscle GAA decreased and creatine increased with dosage. This gives evidence dietary GAA is effectively being converted to creatine and taken up by the tissues, as well, the conversion of muscle GAA to creatine may be occurring within the tissue which would contribute to the increase in muscle creatine concentrations. All in all, GAA supplementation in poultry can aid in feed conversion, has positive effects on carcass characteristics, allows for Arg sparing, and may aid in stress-inducing situations.

Swine

The earliest work found using GAA supplementation in swine was published in 2012 examining the effects on growth performance, antioxidation, and quality of meat in pigs (Wang et al., 2012). Prior to this, only CMH supplementation was evaluated for its effects. Wang et al. (2012) examined the effects of 4 levels of GAA (0, 0.8, 1.2, 2.0 g/kg feed) in 144 crossbred growing-finishing pigs (45 kg) fed a corn-based diet with soybean meal and rice bran as other prominent components for 54 days. No differences were observed in the growth performance of the animals, but there were effects of GAA on meat quality and antioxidation capabilities. A linear increase in pH was measured in the longissimus dorsi muscle (LD) with control at a pH of 5.78 and 2.0 g GAA/kg at 5.99. However, notably, there was a quadratic tendency in pH as well due to 0.8 g GAA/kg having the highest pH of 6.22 (Wang et al., 2012). Greater pH can indicate a delay in post-mortem glycolysis occurring due to the greater availability of ATP being regenerated from PCr. With less glycolysis, less lactic acid is produced, and the pH remains

higher for a longer period of time. This can aid in enhancing the quality of the meat as water retention can be greater. This was evidenced by a decrease in drip loss percentage in GAA-supplemented groups compared to control with the least loss observed in 0.8 g GAA/kg followed by 1.2 g GAA/kg (Wang et al., 2012). Similarly, 0.8 g GAA/kg had the lowest shear force and yellow pigment compared to the other treatment groups, followed by 1.2 g GAA/kg. Each of these differences provide a better meat quality and can lead to a better eating experience for consumers.

Antioxidant capacity of pigs supplemented with GAA increased compared to control animals (Wang et al., 2012). In plasma, compared to control, 0.8 g GAA/kg increased catalase and glutathione peroxidase (these convert hydrogen peroxide, a reactive oxygen species, to hydrogen and water) accompanied by a reduction in malondialdehyde, a terminal product of lipid peroxidation. Total antioxidant capability of the body was greatest for 1.2 g GAA/kg followed by 0.8 g GAA/kg compared to the other treatments. In the LD, 0.8 g GAA/kg led to the greatest increases in superoxide dismutase (enzyme involved in the dismutation of superoxide anion preventing formation of hydroxyl radicals), glutathione peroxidase, and catalase compared to other treatments, with comparable results for 1.2 g GAA/kg. In the LD, 0.8 g GAA/kg led to the lowest malondialdehyde concentration and followed the 1.2 g GAA/kg group in total antioxidant capability. The results of Wang et al. (2012) demonstrated greater antioxidative status in pigs supplemented GAA, particularly at 0.8 and 1.2 g GAA/kg feed. This and the results observed in meat quality could result in a better meat product with GAA supplementation. From this, most work in the following years primarily focused on the effects of GAA on meat quality.

McBreairty et al. (2015) took a different approach from the focus of early work in swine and examined how GAA affected creatine stores as well as the creatine and other metabolic

pathways in the body when supplemented to Yucatan miniature pigs. Animals were fed a standard grower diet low in creatine and allocated to a control treatment or equimolar supplementation of either 0.157 g GAA/kg feed/d or 0.2 g CMH/kg feed/d. On the last day of treatment (day 18 or 19) a single pulse dose of labeled Met was intravenously infused to measure effects on methylation in the body. After 30 minutes was allowed to reach a constant rate of incorporation, pigs were slaughtered for tissue samples. Body weight gain did not differ between treatment groups, but there were differences in metabolite concentrations in tissue and plasma. Supplementation of GAA increased liver and muscle creatine concentrations and liver, muscle, kidney, and plasma GAA concentrations compared to control. Creatine monohydrate supplementation was intermediate in all instances except plasma GAA where it was similar to the control. Compared to control, both supplemental groups increased kidney creatine similarly. The authors noted that tissues with originally low creatine concentrations such as the liver and kidney had a greater response than those with naturally high concentrations like skeletal muscle. Supplemental CMH groups had 2.4 times the plasma creatine concentrations of control with GAA having half the response as CMH. Possibly, greater creatine synthesis is occurring within extra-hepatic tissues with increased GAA supply. This is evidenced by greater creatine concentrations in these areas even with greater plasma creatine concentrations that would down regulate creatine transportation into the cell. No differences were measured in the heart or brain for either substance. The authors reference data indicating some GMAT activity, converting GAA to creatine, has been measured in the muscle of young pigs (Brosnan et al., 2009). In a newer article, GMAT expression was actually increased in the semitendinosus muscle (ST) by CMH supplementation, with supplemental GAA intermediate to the control, giving support to this claim (Li et al., 2018).

Measurement of Met incorporation exhibited faster methylation of GAA to creatine with dietary GAA (McBreairty et al., 2015). This was accompanied by lower incorporation rate of Met into phosphatidylcholine and protein in general. A reduction in other important methylation reactions is evident in this data. As expected with more methylation to creatine, hepatic SAM concentration was reduced with GAA supplementation. However, interestingly, hepatic SAH concentration was not different among treatments as it was suspected to be readily hydrolyzed to Hcy; this was evidenced by greater plasma Hcy concentrations compared to the other treatment groups (McBreairty et al., 2015). The addition of GAA to the diet increased concentrations in the body of both GAA and creatine similar or greater than CMH supplementation. In addition, evidence was provided that shows GAA methylation uses a major portion of SAM, and issues may arise in other reactions without proper supply of methyl groups.

In 2016, weaned piglets and fattening hogs were added to the European Union's regulation on GAA allowing use in a commercial setting at 0.6-1.2 g/kg feed (Regulation (EC) No 1831/2003; European Commission, 2016). A large amount of published work following this approval focused on the growth of pigs in the post-weaning phases, effects on carcass traits, and changes in metabolites in the body. Body weight was greater compared to control at day 60, 90, 120, and 180 of age in a 150-day feeding study with 1.2 g GAA/kg fed in the grower and finisher phases to 360 crossbred pigs (7.17 kg) of an equal mix of sex (Jayaraman et al., 2018). Average daily gain and G:F were improved with 1.2 g GAA/kg in each phase of diet (pre-starter, starter, grower, and finisher) and in the overall study compared to control, with 0.8 g GAA/kg intermediate. He et al. (2018) supplemented graded levels of GAA (0, 0.3, 0.6, 0.9, and 1.2 g/kg feed) to 180 crossbred weaned pigs (33.61 kg), of an equal sex ratio, fed a corn/soybean meal-based diet for 98 days. The grower, finisher 1, and finisher 2 phases of diet were each 35 days.

After the treatment period, 30 pigs (6 per treatment) were slaughtered to obtain carcass data. During the grower phase, there was a linear decrease in ADFI with increasing GAA. Numerically, however, the reduction was from 0.3-0.9 g GAA/kg as 1.2 g GAA/kg had intakes similar to control. Supplementation of GAA increased G:F during the finisher 1 and 2 phases with 0.3 g GAA/kg having (finisher 1) or tending to have (finisher 2) the greatest improvement over control. Over the total 98 days, G:F was linearly improved with increasing GAA, but responses were fairly similar between supplementation groups. The 0.3 g GAA/kg treatment yielded the greatest improvement in performance in He et al. (2018). This is corroborated in a study where 0.3 g GAA/kg increased final body weight and ADG compared to control and 0.6 g GAA/kg, with supplementation of 0.15 and 1.2 g GAA/kg intermediate (He et al., 2018). Similar improvements in cellular concentrations at 0.3 g GAA/kg are described below. On a wheat/barley-based diet, supplementation of weaned pigs (6.20 kg) with 1.2 g GAA/kg feed increased ADFI while minimally increasing ADG numerically, resulting in a greater F:G compared to control over a 50-day feeding trial (Pedersen et al., 2021). The 50 days were split into 4 feeding phases: diet 1 (day 0-14), diet 2 (day 15-21), diet 3 (day 22-28), and diet 4 (day 29-50). Milk powder was included at 11% of the diet during diet 1 and fish meal was included at 6.8, 3.8, and 1.9% in diet 1, 2, and 3, respectively. With 1.2 g GAA/kg in the diet, pigs had greater ADG during diet 3 and 4 compared to control, indicating GAA may be useful during the later finisher stages of growth. However, the control group had greater body weight on day 14 and 21 as well as better F:G during each phase as well as the total feeding period (Pedersen et al., 2021). The minimal effect of GAA on performance may have been due to the inclusion of milk powder and fishmeal (creatine sources) in the early phases. Overall, GAA supplementation

appears to improve growth performance in growing-finishing pigs in most instances, with 1.2 g GAA/kg being the effective dose in most studies.

Jayaraman et al. (2018) examined if the length of supplementation alters the effects of GAA supplementation in the finisher phase. Supplementation of 1.2 g GAA/kg was provided with a corn/soybean meal finisher diet to all animals except the control group for 60, 40, or 25 days prior to slaughter. Body weight, ADG, and G:F was greatest in the 60-day group, with similar results in the group fed for 40 days. The 25-day group did not differ from control. One-hundred forty-four pigs (36 pigs/treatment; equal barrows and gilts) out of the total 1,440 were slaughtered after the 60-day trial (day 120 to 180 of age). With no differences in carcass weight, the 60-day group had a greater lean meat yield, by weight and content percentage, as compared to the control and 25-day supplementation group. The 60-day group had the least backfat statistically and visually compared to control, the other 2 supplementation groups were intermediate. In contrast, compared to control, barrows (90 kg) supplemented 1 g GAA/kg feed for only 15 days prior to slaughter increased ADG by 136 g/d, with 0.8 g CMH/kg feed having an intermediate response of 36 g/d over control (Li et al., 2018). However, ADFI was greater with GAA than control and CMH by ~400 g/d. These 2 studies show conflicting data of the effect length of GAA supplementation has on the magnitude of improvement in finishing performance of pigs.

The effects of GAA supplementation on pork carcass traits have shown promising results. Although there were no differences in carcass weight, lean meat yield by weight was ~1.5 kg greater and lean content percentage was ~1.5% greater for pigs supplemented with 1.2 g GAA/kg for 150 days, relative to control pigs (Jayaraman et al., 2018). Back fat was less when measured on the animal and carcass with 1.2 g GAA/kg than control, with 0.8 g GAA/kg intermediate. In

barrows fed a corn/soybean meal-based finisher diet with treatments consisting of the control diet, 0.8 g CMH/kg feed, and an equimolar 1.0 g GAA/kg feed, positive results were measured in meat quality in both the LD and ST (Li et al., 2018). After 45 minutes post-mortem, the GAA treatment had greater pH, similar to CMH supplementation, in the LD and ST compared to control, but there were no differences amongst treatments 24 hours post-mortem (Li et al., 2018). A greater pH post-mortem indicates a reduction in glycolysis occurring after death when no oxygen is supplied to the tissues, as described above. This is directly linked to meat quality as there is a lesser chance of pale, soft, and exudative meat, especially in pork. In accordance, without the drop in pH, water retention is maintained and there is less drip loss. In this study, GAA and CMH decreased drip loss in the LD and only CMH decreased drip loss in the ST (Li et al., 2018). In addition, the expression of calpain 1, which plays a major role in the tenderness of the meat through rate and extent of proteolysis, was increased in the LD and ST by GAA, similar to CMH, compared to control (Li et al., 2018). Supplementation of GAA has not increased carcass size but does have positive effects on pork carcass traits and factors that affect meat quality.

When examining metabolites in the body, He et al. (2018) observed serum GAA and liver creatine tended to increase with 0.3 g GAA/kg compared to control, but linearly decreased with increasing GAA up to 1.2 g GAA/kg after 98 days of supplementation. Serum CK tended to increase at day 70 with 1.2 g GAA/kg, followed by 0.3 g GAA/kg, but was not different at any other time point in the study. In addition, serum ATP linearly increased 0.20 mmol/L from control to 1.2 g GAA/kg and 0.15 mmol/L from control to 0.3 g GAA/kg. In the muscle, ATP was increased most by supplementation of 0.3 g GAA/kg compared to the other treatments. In another study, GAA (1.0 g/kg feed) increased creatine concentration in the LD and liver and PCr

in both the LD and ST compared to control, with increases being similar to GAA for supplementation with an equimolar amount of CMH (Li et al., 2018). An increase in creatine and/or PCr would allow greater regeneration of ADP to ATP, explaining the increase in ATP concentration. Also, changes in gene expression have been measured with GAA supplementation. Decreases in AGAT expression in the liver with 1.2 g GAA/kg (He et al., 2018) and in the kidney with 1.0 g GAA/kg (Li et al., 2018) have been measured in comparison to control groups. Expression of GMAT was decreased in the liver with 1.2 g GAA/kg (He et al., 2018) but increased with 1.0 g GAA/kg compared to control (Li et al., 2018). Comparatively, CMH supplementation increased kidney GMAT expression compared to control, with an intermediate response with 1.0 g GAA/kg supplemented. The creatine transporter expression was also increased in the LD, liver, and kidney with 1.0 g GAA/kg compared to control (Li et al., 2018). During the grower-finisher phase, GAA has an effect on the creatine pathway in many places in the body.

In recent years, research has expanded beyond the grower-finisher phase when examining growth performance of swine with GAA supplementation. Mendonça et al. (2019) examined if maternal supplementation of GAA would have any effect on their progeny and then if further GAA supplementation in the progeny would affect growth performance. According to authors, highly prolific genetic lines are experiencing issues with low growth rates in progeny resulting in low body weight later in life. They believe the Arg sparing capabilities of GAA could possibly aid in the growth of piglets in these lines *in utero* as well as during postnatal growth. Eighty multiparous sows (3^{rd} or 4^{th} litter; 192 kg) began treatments of either a control gestational and lactation diet or diets supplemented with 1.0 g GAA/kg feed 24 hours after insemination. Piglets were cross fostered within 48 hours to even piglet sizes amongst sows and received no creep feed

during lactation, then were weaned on day 23. After weaning, piglets were sorted by weight (light weight average = 4.9 kg, heavy weight average = 6.8 kg), and provided treatments in a 2 x 2 factorial (sow diet x nursery diet). Treatments in the nursery diet included the control or supplementation of 1.0 g GAA/kg feed. Piglets were then fed for 40 days to day 63 of life. There were no differences in performance between treatments at any stage during the trial. The authors conducted an economic analysis of providing GAA in this manner and observed a tendency for GAA to be economically valuable during the first 2 nursery phases (day 23-28 and 29-36 of life), but in the last 2 phases (day 37-53 and 54-63) and overall, the control diet had more economic value than diets with added GAA to the diet (Mendonça et al., 2019). From a growth and economical aspect GAA supplementation does not improve sow performance and early growth in piglets.

Overall, the research conducted in swine on GAA supplementation has determined many important effects that can enhance production. There have been improvements in antioxidation capacity and creatine status of these animals. Supplementation during the grower-finisher phase has increased performance, but improvements have varied among studies. Lastly, many factors affecting meat quality have been altered by GAA supplementation such as delaying glycolysis, decreasing drip loss and reducing yellowness, resulting in a more desirable product for consumers.

GAA Supplementation in Ruminants

Effects of GAA in ruminants have only recently been of major interest. It is not approved for use in the United States or Europe, but it is unknown if approval is granted elsewhere. The first work to appear was published in 2020 by Ardalan et al. (2020). As little is known about how dietary GAA in ruminants may differ from other animals, the effects on metabolism in the body

and how GAA alters growth performance as well as carcass and meat qualities will be examined from the available literature.

Metabolism

With post-ruminal infusion in heifers (520 kg), Ardalan et al. (2020) observed a linear increase in plasma GAA concentrations with increasing GAA supplementation (0, 10, 20, 30, and 40 g GAA/d), but there was less of an increase when 12 g Met/d was also infused, compared to methionine deficient heifers. Plasma creatine was also increased up to 30 g GAA/d, but 40 g GAA/d was similar to control. As expected, plasma creatinine concentrations were linearly increased with increasing GAA. In another study with abomasal infusions in heifers (181 kg; 0, 7.5, 15 g GAA/d), infused GAA linearly increased plasma GAA and creatine concentrations, however there was no difference in plasma creatinine in this study (Ardalan et al., 2021). In addition, with the same amounts as the previous study but in steers (256 kg), Speer et al. (2022) observed similar increases in plasma creatine concentrations in response to GAA supplementation, but no difference in GAA or creatinine concentrations. These results indicate GAA is absorbed by the small intestine and converted into creatine.

Renal reabsorption of GAA increased with increasing GAA up to 40 g/d infused abomasally when heifers were methionine deficient but decreased with GAA supplementation when heifers were supplemented with 12 g/d Met (Ardalan et al., 2020). This effect was not observed in another study with 6 g Met/d compared to a control group that was methionine deficient (Ardalan et al., 2021). Renal reabsorption of creatine has demonstrated opposing responses between studies (Ardalan et al., 2020; Ardalan et al., 2021). Even with some evidence of increased renal reabsorption, there are greater amounts of GAA and creatine in urinary excretion with post-ruminal GAA supplementation compared to control groups. Ardalan et al.

(2020) measured greater concentrations of urinary GAA and creatine with increasing GAA up to 40 g/d. This is corroborated in part by Speer et al. (2022) who observed a linear increase in urinary GAA and tendency for a linear increase in urinary creatine concentrations from 0 to 15 g GAA/d. However, there was no difference between treatment groups for urinary creatinine. Ardalan et al. (2021) measured no differences in urinary concentrations of GAA, but noted very low GAA concentrations, possibly indicating most was converted to creatine and there was no excess to excrete.

Because GAA methylation uses a large amount of available Met in the body, it is important to understand how supplementation might affect the Met pool. After 4 hours of continuous infusion of 2 sources of labeled Met together, supplemental GAA (0, 7.5, 15 g/d) and Met (0 or 6 g/d) had an interaction to linearly increase the rate of carboxyl carbon- and methyl group-labeled Met flux when no Met was provided (slightly methionine deficient) compared to linearly decreasing both labeled Met source flux rate when Met was infused (Ardalan et al., 2021). In opposition, in steers fed adequate amounts of Met, GAA tended to linearly increase Met flux when 5 g Met/d was additionally supplemented compared to control (Speer et al., 2022). This shows alteration in the availability of Met in the body for reactions when GAA is supplemented in conjunction with Met in various states of methyl deficiency. A similar pattern of increase and decrease, without and with Met, respectively, was measured in protein synthesis and degradation (Ardalan et al., 2021). There was no effect of GAA on transsulfuration, remethylation or Hcy production. Because GAA provision increased, which was expected to increase GAA methylation to creatine, methylation reactions besides conversion of GAA to creatine would decrease if total methylation reactions remained the same. The 6 g Met/d group had increased transsulfuration, and reduced remethylation, compared to control, but no

differences in Hcy production were observed (Ardalan et al., 2021). Exogenous GAA appears to alter Met flux when Met is also provided.

Changes in plasma amino acid concentrations can give some insight into what is occurring in other processes in the body. In Ardalan et al. (2020), plasma Arg concentrations were linearly increased with up to 40 g GAA/d, whereas Orn was increased with 10 g GAA/d followed by 20 g GAA/d, indicating Arg sparing for other pathways because less Arg was required for synthesis of GAA. Similarly, Speer et al. (2022) determined 7.5 g GAA/d increased plasma Arg and tended to increase Orn (with Met supplementation) and citrulline compared to other treatment groups, indicating an effect at varying points of the urea cycle. Homocysteine was increased by GAA supplementation in multiple studies but was not increased when Met was also supplemented (Ardalan et al., 2020; Ardalan et al., 2021). Also, Ser, needed for transsulfuration, decreased with GAA alone, but increased when Met was infused in conjunction (Ardalan et al., 2021). Other amino acids such as Glu, Trp, Tyr, Thr, and Asn were greater with GAA than control with varying increases at different GAA provision amounts (Ardalan et al., 2020; Ardalan et al., 2021), and GAA supplementation also tended to increase Ile and Gln (Speer et al., 2022). In addition, His, Asp, and Leu decreased (Ardalan et al., 2020; Ardalan et al., 2021) and Ala tended to decrease with GAA supplementation (Speer et al., 2022). With post-ruminal provision of GAA, amino acid concentrations in plasma have been altered indicating an effect on various pathways in the body.

Nitrogen retention is a good way to measure protein deposition from the difference in nitrogen provided (fed and infused) and nitrogen excreted (urinary and fecal). Steers (161 kg) were supplemented by abomasal infusion with 0 or 6 g Met/d and 0, 7.5, or 15 g GAA/d (Ardalan et al., 2021). As expected, post-ruminal GAA infusion linearly increased nitrogen

intake, but also linearly increased urinary nitrogen. Fecal nitrogen increased with 7.5 g GAA/d. The interaction of these 2 treatments (Met x GAA) led to the tendency of nitrogen retention to be Met dependent. Methionine-deficient cattle probably would not be able to sustain increased creatine synthesis or protein deposition with added GAA. In a study with the same amounts of GAA, there were no differences among treatments for DM or organic matter digestibility (Speer et al., 2022). Methionine abomasally infused at 5 g Met/d once again increased nitrogen intake and decreased urinary nitrogen resulting in greater nitrogen retention compared to steers that did not receive supplemental Met. Again, GAA of the same amounts as above linearly increased nitrogen intake and urinary nitrogen. However, the loss was slightly greater than the intake, leading to a numerical decrease in nitrogen retention. When compared to creatine supplementation, 15 g GAA/d provided post-ruminally increased nitrogen retention compared to the control (Grant et al., 2021). There is a definite need for more work assessing nitrogen retention responses to GAA supplementation.

Bioavailability

To apply supplementation of GAA to ruminants practically, it will need to be fed. Therefore, the bioavailability to the animal is needed to determine the amount required to be fed to reach an effective level of supplementation. Speer et al. (2020) either ruminally or abomasally infused 10 or 20 g GAA/d to steers (208 kg) on an even roughage:concentrate diet and compared responses to a control receiving only water infusion with the basal diet. There was no difference among treatments in plasma GAA, but plasma creatine concentrations linearly increased with abomasal infusion and tended to linearly increase with ruminal infusion. Elevated creatine concentrations suggest GAA was absorbed when provided post-ruminally, and the unchanged plasma GAA concentrations suggest a ready conversion of GAA to creatine. Similarly, there was

no difference in urinary GAA, but urinary creatine linearly increased with both infusion sites. Based on plasma and urinary creatine concentrations, ruminal escape of GAA was estimated to be near 50%, meaning double the amount will need to be fed to provide amounts of GAA determined to be effective when infused post-ruminally.

Dietary Supplementation

The addition of GAA to ruminant diets appears to have many effects on growing animals that could be advantageous in growth performance of feeding cattle for meat production. Li et al. (2020) observed a slight increase in dry matter intake (DMI) with 0.6 and 0.9 g GAA/kg DM compared to control. Liu et al. (2021a) measured a small 2% improvement in body weight with 0.6 g/kg DM compared to control by 30 days of supplementation but observed roughly 20 kg heavier body weight on day 60. A similar 20-kg advantage was measured on day 60 by Li et al. (2020) with 0.6 and 0.9 g/kg DM . With an additional 30 days of supplementation, at day 90 a 30-kg difference was measured between those groups and control (Li et al., 2020). Another study observed a 10-kg gain of body weight over control with 0.6 g GAA/kg DM (Liu et al., 2021b). Average daily gain has increased with 0.6 g GAA/kg DM by ~0.4 kg/d in various studies in bulls averaging 400 kg over control groups supplemented for 80 (Liu et al., 2021a; Liu et al., 2021b) or 104 days (Li et al., 2020). Li et al. (2021) supplemented graded levels of GAA (0, 0.5, 1.0, 2.0, and 4.0 g/kg DM) to bulls (350 kg) for 42 days and observed ADG was 0.4 kg/d greater for bulls fed 2.0 g GAA/kg DM compared to control bulls, with all other GAA treatments intermediate. Improvements in F:G matched changes in ADG because DMI was not affected strongly by treatment. Similarly, F:G was improved 10% with 0.6 g GAA/kg DM compared to the control treatment, and 0.9 and 0.3 g GAA/kg DM were intermediate (Li et al., 2020).

Liu et al. (2021a) and Liu et al. (2021b) examined the addition of betaine and coated folic acid to 0.6 g GAA/kg DM, respectively, when fed to Angus bulls (~430 kg). Both betaine and folic acid had individual effects on body weight, ADG, and G:F. With addition of 0.6 g betaine/kg DM to the GAA treatment, advantages of 28 kg in body weight at day 60, 0.4 kg/d in ADG, and a 27% improvement in feed efficiency were observed compared to control, but provision of only betaine or GAA were similar statistically to the combination of the 2 treatments (Liu et al., 2021a). With 0.35 g coated folate/kg DM and GAA, ADG increased 0.36 kg/d (similar to the increase of betaine and GAA) from 0-30, 30-60 days, and the overall study (Liu et al., 2021b). The combination of folate and GAA had a 25% improvement of G:F over control, but individual GAA and coated folate treatment groups were similar to each other. Growth performance in cattle appears to be enhanced with supplemental GAA with the possibility of greater improvement with the addition of betaine or coated folic acid.

Digestibilities of DM, OM, protein, and fiber were increased with added dietary GAA (Li et al., 2020; Liu et al., 2021a; Liu et al., 2021b). Along with improved digestibility, greater ruminal concentrations of volatile fatty acids have been measured with an associated decrease in ruminal pH with 0.6 g GAA/kg DM compared to control groups (Li et al., 2020; Liu et al., 2021a; Liu et al., 2021b). Ruminal pH tended to be lowest when coated folic acid was supplemented in addition to GAA (Liu et al., 2021b). In a 2 x 2 factorial study, with 0 or 0.6 g GAA/kg DM and 0 or 0.6 g betaine/kg DM, Liu et al. (2021a) observed total bacterial and protozoal populations increased with 0.6 g GAA/kg DM compared to control. The combination of betaine and GAA resulted in the greatest populations of *Ruminococcus flavefaciens* and *Fibrobacter succinogenes*, 2 prominent cellulolytic microbes in the rumen, and a tendency for the greatest protozoal population. Li et al. (2020) also measured an increase in ruminal bacteria

with 0.6 g GAA/kg DM as well as fungi with 0.9 g GAA/kg DM compared to control, but total protozoa were linearly decreased by these amounts of GAA. Increases above control in many cellulolytic enzymes were measured with supplemental GAA, as well as an increase in protease (Li et al., 2020; Liu et al., 2021a). However, a decrease in ruminal acetate:propionate has been measured compared to control (Li et al., 2020), but concentration of acetate and ammonia nitrogen tended to be greater with the combination of GAA and betaine (Liu et al., 2021a). Ruminal ammonia nitrogen concentration has been shown to increase (Liu et al., 2021a) and decrease (Li et al., 2020) with GAA indicating the possibility of greater ruminal protein degradation and/or microbial synthesis.

When examining metabolites in the blood of cattle, there are a few notable effects of GAA supplementation. In all studies referenced above in this section, plasma creatine concentrations were increased with 0.5 and 1.0 g GAA/kg (Li et al., 2021), 0.6 g GAA/kg (Liu et al., 2021a; Liu et al., 2021b), and 0.3 to 0.9 g GAA/kg DM (linear increase; Li et al., 2020). A quadratic response was measured in serum Hcy concentrations with 0.5 and 4.0 g GAA/kg DM resulting in a decrease, whereas 2.0 g GAA/kg DM had concentrations greater than control (Li et al., 2021). As would be expected, a combination of GAA and coated folic acid increased plasma and hepatic creatine concentrations compared to control (Liu et al., 2021b). Other amino acids involved in GAA/creatine synthesis (Arg and Met) did not differ from control (Li et al., 2020; Liu et al., 2021a; Liu et al., 2021b). There have been no differences in blood urea nitrogen and ammonia nitrogen as a result of GAA supplementation (Li et al., 2020; Li et al., 2021). Similar to pigs, increases in superoxide dismutase and reduced glutathione (GSH) were measured compared to control, resulting in a greater reduced to oxidized glutathione ratio meaning greater antioxidant capacity in the animal (Li et al., 2021).

Guanidinoacetic acid has improved meat quality in beef. Increases in muscle glycogen, creatine, CK, and laminin concentrations was measured in the LD with 2.0 g GAA/kg DM compared to control (Li et al., 2021). A decrease in lactate dehydrogenase was measured in the LD and this would result in lactate (produced by glycolysis in the anaerobic state of meat) concentrations remaining elevated, which would negatively feedback to slow glycolysis. This, with the added energy from glycogen and creatine, would potentially decrease pH for a period of time; however, this was not observed in either the LD or ST. Drip loss was reduced by GAA supplementation by ~0.5% in the LD and ST compared to control, indicating water retention was enhanced. In addition, a tendency for less Type IV collagen was measured when GAA was supplemented, leading to a tendency for decreased shear force in the ST, meaning greater tenderness for the consumer. Supplementation of GAA decreased lightness in the LD, but redness and yellowness were increased (Li et al., 2020). Overall, GAA had a positive effect on quality of beef.

In lambs, the effects of GAA supplementation have been examined in growth performance and metabolite concentrations as well as a more in depth look into carcass characteristics. Zhang et al. (2022b) observed no difference in final body weight, ADG, or G:F when 0, 0.5, 1.0, or 1.5 g GAA/kg DM was supplemented to Kazkh male lambs (27 kg) for 100 days. These lambs were fed an all-plant based diet, meaning creatine/GAA was not provided in the basal diet. Supplementation of GAA had a quadratic effect on DMI with 0.5 g GAA/kg DM consuming the least with control and 1.5 g GAA/kg DM consuming the most DM. A quadratic effect was measured in plasma GAA concentrations with 0.5 g GAA/kg DM leading to the greatest increase, followed by 1.0 g GAA/kg DM then 1.5 g/kg DM. This could be due to a similar quadratic increase in GAA concentrations in all portions of small intestine mucosa and

portal vein indicating absorption in the gut. Transporters SLC6A8 and SLC6A6 are reported to be the creatine transporter and possible GAA transporter, respectively. The creatine transporter, SLC6A8, is thought to have some capability of GAA transport as well. There was a cubic response in the jejunum for SLC6A6 (GAA transporter) with 1.0 g/kg DM yielding the greatest expression followed by control. Providing 1.5 g/kg DM resulted in the lowest expression compared to other treatments. Supplementation tended to linearly decrease SLC6A8 (creatine transporter) in the jejunum and linearly decreased it in the ileum. Authors suggested the greater levels of dietary GAA may have inhibited and limited expression of these transporters, which would coincide with the lesser concentrations in the portal vein. Hepatic GMAT expression or creatine concentration in the hepatic tissue did not differ amongst treatments, but creatine concentration in the hepatic vein plasma were greater with 0.5 g GAA/kg DM, followed by 1.0 g/kg DM, with 1.5 g GAA/kg DM being similar to control. However, no difference was observed in plasma creatine collected from the jugular vein (Zhang et al., 2022b).

In the quadriceps femoris, GAA linearly increased ATP and CK concentrations, whereas there was a tendency for creatine to decrease with 0.5 g GAA/kg DM being the lowest compared to other treatments (Zhang et al., 2022b). Similarly, ATP increased in the LD, but also PCr and ADP. When GAA is supplemented, converted to creatine, and taken up into the tissues there is an increase in the capacity of the CK system. The additional creatine is phosphorylated to make PCr which then can phosphorylate ADP to ATP resulting in more energy availability within the tissue. An increase in all components is needed to yield this result. The ratio of PCr to Cr was greatest for 1.0 g GAA/kg DM over 0.5 g GAA/kg DM, with control and 1.5 g/kg DM intermediate (Zhang et al., 2022b). These results indicate creatine was converted to PCr and used for ATP regeneration.

Zhang et al. (2022a) examined the effects of 2 levels of ruminally protected methionine (RPM; 0.6 or 0.8 g/kg DM) when added to 0.8 g GAA/kg DM (RPM + GAA treatments = 0.6 g RPM + 0.8 g GAA/kg DM or 0.8 g RPM + 0.8 g GAA/kg DM) in castrated male Tan lambs (19 kg) compared to a control group only receiving the basal diet for 90 days. This would determine if additional methyl groups would aid in the enhancement of performance by GAA supplementation. Supplementation of 0.6 g RPM + 0.8 g GAA/kg DM increased final body weight just over 1.5 kg, ADG for each 30 days and whole trial (~20 g/d), and G:F by 8% compared to control. In comparison to cattle, blood urea nitrogen was decreased with 0.6 g RPM + 0.8 g GAA/kg DM compared to control. In all measurements discussed above, 0.8 g RPM + 0.8 g GAA/kg DM was intermediate. The 0.8 g RPM + 0.8 g GAA/kg DM supplemental combination also had increased muscle glycogen content.

Compared to control, dressing percentage was greater by 4% for 0.6 g RPM + 0.8 g GAA/kg DM, with 0.8 g RPM + 0.8 g GAA/kg DM intermediate (Zhang et al., 2022a). As well, net meat weight as a percentage of carcass weight was greater for 0.6 g RPM + 0.8 g GAA/kg DM than for control and 0.8 g RPM + 0.8 g GAA/kg DM. Increasing RPM from 0.6 g/kg DM to 0.8 g/kg DM increased crude protein content of the LD, which was assumed due to greater protein synthesis with the added Met. The 0.6 g RPM + 0.8 g GAA/kg DM treatment group increased intramuscular fat. This enhances the quality of the meat and the eating experience for the consumer. In addition, 0.6 g RPM + 0.8 g GAA/kg DM had a higher pH 45 minutes post-mortem and tended to 24 hours post-mortem, while being similar to the pH of 0.8 g RPM + 0.8 g GAA/kg DM at 48 hours. Accompanied with the elevated pH, the 0.6 g RPM + 0.8 g GAA/kg DM had increased water holding capacity, less cooking loss, and decreased shear force. These are all factors that increase meat quality (discussed above). The 0.8 g RPM + 0.8 g GAA/kg DM

treatment group had decreased brightness of the meat at 0-, 24-, and 48-hours post-mortem while also having greater redness at slaughter that increased in 24 hours and greater yellowness at 24 hours compared to control. The 0.6 g RPM + 0.8 g GAA/kg DM increased redness and yellowness at 24 hours similarly to 0.8 g RPM + 0.8 g GAA/kg DM (Zhang et al., 2022a). Overall, 0.6 g RPM + 0.8 g GAA/kg DM has the most benefit for growth performance and meat quality in lambs.

Li et al. (2022) supplemented either 0 or 0.9 g GAA/kg DM to crossbred ram lambs (25 kg) fed a high concentrate diet for 70 days prior to slaughter. An increase in final body weight as well as carcass and total meat weight were measured with GAA compared to control. The area of the LD was increased by GAA supplementation, as was the frequency of large diameter muscle fibers. In the LD, crude protein percent, pH at 24 hours post-mortem, and water holding capacity were greater in GAA-supplemented lambs. This is most likely due to the greater CK and decrease in lactate dehydrogenase in the muscle, but there was no difference in muscle glycogen. Authors suspect due to the Arg sparing effect of GAA there was more Arg available for protein synthesis, but this was not directly assessed. Such as in cattle and pigs, oxidative status was enhanced by GAA through increases in catalase and glutathione peroxidase whereas malondialdehyde was reduced by GAA compared to control.

Taking a deeper look into muscle growth with supplemental GAA, the Akt/mTOR (target of rapamycin) signaling pathway and associated proteins were examined by Li et al. (2022). Supplementation of 0.9 g GAA/kg DM increased LD concentrations of IGF-1, phosphorylated Akt, and phosphorylated mTOR, but there were no differences between GAA and control for total Akt and mTOR proteins. Still, elevation and activation of these proteins can promote skeletal growth which could consequently result in the greater body weight shown in this study.

In addition, a decrease in myostatin expression and concentration was measured. Myostatin regulates myocellular growth by inhibition, so a decrease would indicate greater muscle growth. Also, FOXO1 (Forkhead Box O1) when phosphorylated by Akt prevents degradation of proteins. Supplemental GAA increased the concentration of phosphorylated FOXO1, which might reduce protein degradation. *In vitro*, GAA elevated myoblast differentiation as well as heightened activation of Akt/mTOR pathway compared to control, as was evidenced above *in vivo* (Li et al., 2022). The net result of GAA supplementation in lambs is stimulation of protein synthesis and reduction in protein degradation for a subsequent heavier animal with better meat quality.

Supplementation of GAA appears to have a positive effect on growth performance, nutrient digestion, and meat quality, but the studies discussed have a small number of animals per treatment. Therefore, further evaluation with a larger number of observations would allow a clearer picture of the effect GAA supplementation has in ruminants.

Conclusion

Guanidinoacetic acid supplementation improves production of livestock, including poultry, pigs, and ruminants. This is evidenced by improvements in growth performance, creatine and energy status, as well as antioxidant status. In addition, greater meat yield and meat quality has been shown with supplemental GAA. In comparison to creatine supplementation, GAA appears to yield a greater result in these areas. However, there are still many aspects of production, especially in ruminants, that need to be further researched in metabolism and supplementation of GAA.

Literature Cited

Ardalan, M., E. D. Batista, and E. C. Titgemeyer. 2020. Effect of post-ruminal guanidinoacetic acid supplementation on creatine synthesis and plasma homocysteine concentrations in cattle. J. Anim. Sci. 98:1–9. doi:10.1093/jas/skaa072.

Ardalan, M., M. D. Miesner, C. D. Reinhardt, D. U. Thomson, C. K. Armendariz, J. S. Smith, and E. C. Titgemeyer. 2021. Effects of guanidinoacetic acid supplementation on nitrogen retention and methionine flux in cattle. J. Anim. Sci. 99:1–12. doi:10.1093/jas/skab172.

Ball, R. O., K. L. Urschel, and P. B. Pencharz. 2007. Nutritional Consequences of Interspecies Differences in Arginine and Lysine Metabolism. The Journal of Nutrition. 137:1626S-1641S. doi:10.1093/jn/137.6.1626S.

Brosnan, J. T., and M. E. Brosnan. 2007. Creatine: Endogenous metabolite, dietary, and therapeutic supplement. Annu. Rev. Nutr. 27:241–261. doi:10.1146/annurev.nutr.27.061406.093621.

Brosnan, J. T., E. P. Wijekoon, L. Warford-Woolgar, N. L. Trottier, M. E. Brosnan, J. A. Brunton, and R. F. Bertolo. 2009. Creatine synthesis is a major metabolic process in neonatal piglets and has important implications for amino acid metabolism and methyl balance. J. Nutr. 139:1292–1297. doi:10.3945/jn.109.105411.

Crim, M. C., D. H. Calloway, and S. Margen. 1976. Creatine metabolism in men: Creatine pool size and turnover in relation to creatine intake. J. Nutr. 106:371–381. doi:10.1093/jn/106.3.371.

EFSA, (European Food Safety Authority). 2009. Safety and efficacy of guanidinoacetic acid as feed additive for chickens for fattening. The EFSA Journal. 988:1–30. doi:10.2903/j.efsa.2009.988.

European Commission. 2016. Commission Implementing Regulation (EU) 2016/1768 of 4 October 2016 concerning the authorisation of guanidinoacetic acid as a feed additive for chickens for fattening, weaned piglets and pigs for fattening and repealing Commission Regulation (EC) No 904/2009 (Text with EEA relevance), C/2016/6279. Official Journal of the European Union. doi:https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32016R1768.

FDA, (U. S. Food and Drug Administration). 2021. Code of Federal Regulations, Title 21 - Food and Drugs, Section 573.496 - Guanidinoacetic acid. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=573&showFR=1

Grant, M. S., M. D. Miesner, and E. C. Titgemeyer. 2021. Effects of guanidinoacetic acid, creatine, and choline on protein deposition and creatine status in growing cattle. Kansas Agricultural Experiment Station Research Reports. Vol. 7. doi:10.4148/2378-5977.8032.

He, D. T., X. R. Gai, L. B. Yang, J. T. Li, W. Q. Lai, X. L. Sun, and L. Y. Zhang. 2018. Effects of guanidinoacetic acid on growth performance, creatine and energy metabolism, and carcass characteristics in growing-finishing pigs. Journal of Animal Science. 96:3264–3273. doi:10.1093/jas/sky186.

Jayaraman, B., K. V. La, H. La, V. Doan, E. M. Carpena, M. Rademacher, and G. Channarayapatna. 2018. Supplementation of guanidinoacetic acid to pig diets: effects on performance, carcass characteristics, and meat quality. J. Anim. Sci. 96:2332–2341. doi:10.1093/jas/sky137.

Li, J., L. Zhang, Y. Fu, Y. Li, Y. Jiang, G. Zhou, and F. Gao. 2018. Creatine monohydrate and guanidinoacetic acid supplementation affects the growth performance, meat quality, and creatine metabolism of finishing pigs. J. Agric. Food Chem. 66:9952–9959. doi:10.1021/acs.jafc.8b02534.

Li, S. Y., C. Wang, Z. Z. Wu, Q. Liu, G. Guo, W. J. Huo, J. Zhang, L. Chen, L. Y. Zhang, C. X. Pei, and S. L. Zhang. 2020. Effects of guanidinoacetic acid supplementation on growth performance, nutrient digestion, rumen fermentation and blood metabolites in Angus bulls. Animal. 14:2535–2542. doi:10.1017/S1751731120001603.

Li, X., X. Liu, P. Song, J. Zhao, J. Zhang, and J. Zhao. 2022. Skeletal muscle mass, meat quality and antioxidant status in growing lambs supplemented with guanidinoacetic acid. Meat Sci. 192:1–9. doi:10.1016/j.meatsci.2022.108906.

Li, Z., H. Liang, J. Xin, L. Xu, M. Li, H. Yu, W. Zhang, Y. Ge, Y. Li, and M. Qu. 2021. Effects of dietary guanidinoacetic acid on the feed efficiency, blood measures, and meat quality of Jinjiang bulls. Front. Vet. Sci. 8:1–10. doi:10.3389/fvets.2021.684295.

Liu, C., C. Wang, J. Zhang, Q. Liu, G. Guo, W. J. Huo, C. X. Pei, L. Chen, and Y. L. Zhang. 2021a. Guanididnoacetic acid and betaine supplementation have positive effects on growth performance, nutrient digestion and rumen fermentation in Angus bulls. Anim. Feed Sci. Technol. 276:1–8. doi:10.1016/j.anifeedsci.2021.114923.

Liu, Y. J., J. Z. Chen, D. H. Wang, M. J. Wu, C. Zheng, Z. Z. Wu, C. Wang, Q. Liu, J. Zhang, G. Guo, and W. J. Huo. 2021b. Effects of guanidinoacetic acid and coated folic acid supplementation on growth performance, nutrient digestion and hepatic gene expression in Angus bulls. Br. J. Nutr. 126:510–517. doi:10.1017/S0007114520004341.

Majdeddin, M., U. Braun, A. Lemme, A. Golian, H. Kermanshahi, S. De Smet, and J. Michiels. 2020. Guanidinoacetic acid supplementation improves feed conversion in broilers subjected to heat stress associated with muscle creatine loading and arginine sparing. Poult. Sci. 99:4442–4453. doi:10.1016/j.psj.2020.05.023.

McBreairty, L. E., J. L. Robinson, K. R. Furlong, J. A. Brunton, and R. F. Bertolo. 2015. Guanidinoacetate is more effective than creatine at enhancing tissue creatine stores while consequently limiting methionine availability in Yucatan miniature pigs. PLoS ONE. 10:1–11. doi:10.1371/journal.pone.0131563.

Mendonça, I. B., P. H. Watanabe, B. A. N. Silva, M. M. Boiago, J. C. Panisson, T. S. Andrade, A. C. N. Campos, and M. A. S. P. Mello. 2019. Dietary supplementation of guanidinoacetic acid for sows and their progenies: Performance, blood parameters and economic viability at nursery phase. Livest. Sci. 227:105–110. doi:10.1016/j.livsci.2019.07.011.

Michiels, J., L. Maertens, J. Buyse, A. Lemme, M. Rademacher, N. A. Dierick, and S. De Smet. 2012. Supplementation of guanidinoacetic acid to broiler diets: Effects on performance, carcass characteristics, meat quality, and energy metabolism. Poult. Sci. 91:402–412. doi:10.3382/ps.2011-01585.

Ostojic, S. M. 2016. Guanidinoacetic acid as a performance-enhancing agent. Amino Acids. 48:1867–1875. doi:10.1007/s00726-015-2106-y.

Ostojic, S. M., J. Ostojic, P. Drid, and M. Vranes. 2016. Guanidinoacetic acid versus creatine for improved brain and muscle creatine levels: A superiority pilot trial in healthy men. Appl. Physiol. Nutr. Metabl. 41:1005–1007. doi:10.1139/apnm-2016-0178.

Ostojic, S. M., M. D. Stojanovic, and J. R. Hoffman. 2015. Six-week oral guanidinoacetic acid administration improves muscular performance in healthy volunteers. J. Investig. Med. 63:942–946. doi:10.1097/JIM.0000000000000212.

Pedersen, E. K., J. Dall, C. Amdi, and J. G. Madsen. 2021. Guanidinoacetic acid as a feed supplement offered to weaned piglets. Acta Agric. Scand. A Anim. Sci. 70:145–150. doi:10.1080/09064702.2021.1984560.

Portocarero, N., and U. Braun. 2021. The physiological role of guanidinoacetic acid and its relationship with arginine in broiler chickens. Poult. Sci. 100:1–10. doi:10.1016/j.psj.2021.101203.

Ringel, J., A. Lemme, A. Knox, J. Mc Nab, and M. S. Redshaw. 2007. Effects of graded levels of creatine and guanidino acetic acid in vegetable-based diets on performance and biochemical parameters in muscle tissue. In: Proceedings 16th European Symposium on Poultry Nutrition. Strasbourg, France. p. 387–390. Available from: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.cabi.org/Uploads/animal-science/worlds-poultry-science-association/WPSA-france-2007/68.pdf

Speer, H. F., K. A. Pearl, and E. C. Titgemeyer. 2020. Relative bioavailability of guanidinoacetic acid delivered ruminally or abomasally to cattle. J. Anim. Sci. 98:1–6. doi:10.1093/jas/skaa282.

Speer, H. F., M. S. Grant, M. D. Miesner, and E. C. Titgemeyer. 2022. Effect of guanidinoacetic acid supplementation on nitrogen retention and methionine methyl group flux in growing steers fed corn-based diets. J. Anim. Sci. skac283, https://doi.org/10.1093/jas/skac283

Stead, L. M., K. P. Au, R. L. Jacobs, M. E. Brosnan, and J. T. Brosnan. 2001. Methylation demand and homocysteine metabolism: Effects of dietary provision of creatine and

guanidinoacetate. Am. J. Physiol. Endocrinol. Metab. 281:E1095–E1100. doi:10.1152/ajpendo.2001.281.5.E1095.

Tossenberger, J., M. Rademacher, K. Nemeth, V. Halas, and A. Lemme. 2016. Digestibility and metabolism of dietary guanidino acetic acid fed to broilers. Poult. Sci. 95:2058–2067. doi:10.3382/ps/pew083.

Wang, L. S., B. M. Shi, A. S. Shan, and Y. Y. Zhang. 2012. Effects of guanidinoacetic acid on growth performance, meat quality and antioxidation in growing-finishing pigs. J. Anim. Vet. Adv. 11:631–636. doi:10.3923/javaa.2012.631.636.

Wyss, M., and R. Kaddurah-Daouk. 2000. Creatine and creatine metabolism. Physiol. Rev. 80:1107–1213. doi:10.1152/physrev.2000.80.3.1107.

Wyss, M., and T. Wallimann. 1994. Creatine metabolism and the consequences of creatine depletion in muscle. Mol. Cell. Biochem. 133–134:51–66. doi:10.1007/BF01267947.

Zhang, J. H., H. H. Li, G. J. Zhang, Y. H. Zhang, B. Liu, S. Huang, J. Guyader, and R. Z. Zhong. 2022a. Supplementation of guanidinoacetic acid and rumen-protected methionine increased growth performance and meat quality of Tan lambs. Anim. Biosci. 1–20. doi:10.5713/ab.22.0008.

Zhang, S., C. Zang, J. Pan, C. Ma, C. Wang, X. Li, W. Cai, and K. Yang. 2022b. Effects of dietary guanidinoacetic acid on growth performance, guanidinoacetic acid absorption and creatine metabolism of lambs. PLOS ONE. 17:1–13. doi:10.1371/journal.pone.0264864.

Chapter 3 - Effects of ruminally protected lysine supplementation to limit-fed growing cattle on growing and subsequent finishing performance

Kathryn J. Hazlewood, Madeline S. Grant, Dale A. Blasi, Gary A. Ducharme, and Evan C. Titgemeyer

Abstract

Corn contains relatively low concentrations of Lys and, thus, Lys can become the first limiting for cattle fed corn-based diets (Titgemeyer et al., 1988). Our objective was to determine the effects of supplementing ruminally protected Lys to growing steers fed a corn-based diet during the growing phase and to assess subsequent finishing phase. A 77-d growing phase was implemented using 340 crossbred steers (250 ± 38 kg) allocated to 4 blocks with 8 pens containing 9 to 12 steers in each block. Pens were assigned treatments in a randomized block design. Treatments included: a negative control receiving no supplemental amino acids (control), 3 g/d metabolizable lysine (mLys) from Smartamine ML (Lys-3), 6 g/d mLys from Smartamine ML (Lys-6), and supplemental blood meal (AAdvantage; Perdue Agribusiness, Kings Mountain, NC) at 0.89% of dietary dry matter plus 2 g/d of metabolizable Met provided from Smartamine M. Diets were limit fed at 2.4% of body weight (dry matter basis) once daily and contained 10% dry-rolled corn, 29.5% steam-flaked corn, 40% wet corn gluten feed, 13% hay, and 7.5% supplement. Pens of cattle were weighed weekly to determine performance during the growing phase. Steers were implanted (40 mg trenbolone acetate, 8 mg estradiol) at trial initiation. Blood samples were collected on d 14 and 77 for analysis of plasma amino acid, urea, and haptoglobin concentrations. After the growing phase (77 d), steers were shipped to a commercial feedlot, mixed into 2 finishing pens, fed a common diet without treatment for 185 or 206 d, and slaughtered commercially. Finishing performance was calculated using carcass data from the slaughter facility. During the growing phase, Lys-3 tended to improve average daily gain (quadratic, $P = 0.12$) and efficiency of gain (quadratic, $P = 0.08$) compared to control. Plasma concentrations of Lys ($P > 0.29$) and urea ($P > 0.43$) were not affected by Lys supplementation. During finishing, steers previously fed Lys-6 had numerically greater daily gains than those

previously fed control or Lys-3 (linear, $P = 0.17$). Carcass weights were numerically 7.1 and 3.4 kg heavier (linear, $P = 0.20$) than control for Lys-6 and Lys-3, respectively. Supplementation with BM did not affect performance during the growing or finishing period. Ruminally protected Lys supplementation during the growing phase tended to improve performance of cattle during growing and finishing phases and resulting carcass weight.

Key words: cattle, corn, lysine.

Introduction

Due to deficiencies within microbial protein, methionine (Met) and lysine (Lys) are the most limiting amino acids for cattle fed diets containing little ruminally undegraded protein (RUP; Richardson and Hatfield, 1978). Protein supplementation can increase the supply of limiting amino acids needed for maximum protein deposition and, therefore, weight gain. Corn is a primary feedstuff in cattle diets during the growing and finishing phases. Corn-based protein sources are high in RUP and their inclusion in diets increases amino acids reaching the intestine. Because corn has low Lys but moderate Met contents, its inclusion in the diet at a high percentage can lead to Lys being the first limiting amino acid (Titgemeyer et al., 1988). Free Lys is extensively degraded in the rumen (Cottle and Velle, 1989), so its addition to the diet in an unprotected form is not beneficial. Therefore, ruminally protected Lys (RP-Lys) products such as Smartamine ML (Adisseo, Alpharetta, GA) can be provided to escape rumen degradation and be absorbed in the small intestine (Oke et al., 1986). The objective of this study was to determine the effects of RP-Lys supplementation to growing steers during the growing phase and assess growth performance during the growing phase as well as during the subsequent finishing phase.

Materials and Methods

All procedures involving the use of animals were approved by the Kansas State University Institutional Animal Care and Use Committee.

Animals and Experimental Diets

A 77-d growth trial was conducted using 340 low-risk crossbred steers (250 ± 38 kg initial body weight) at the Kansas State University Beef Stocker Unit, Manhattan, KS. Cattle arrived in 5 loads between July 1 and July 9, 2020, and were sourced from ranches in southern Missouri, southeast Kansas, and northern Arkansas via video auction. Three loads were each maintained as separate blocks, and 2 partial loads with smaller groups of steers were combined into a single block. The 4 blocks had 8 pens each for a total of 32 pens. Steers were stratified by arrival weight, and 9 to 12 steers were allotted to each pen depending on the number of animals in the block (92, 86, 90, and 72). Cattle were housed in outdoor, dirt-packed pens, provided water by automatic waters, and fed in concrete fence-line bunks.

Steers were limit-fed at 2.4% of body weight (BW) once daily (dry matter [DM] basis; Table 3.1). Weekly pen weights were used to calculate feed allocations for the following week. Four experimental treatments were evaluated: 1) a negative control receiving no supplemental amino acids or protein (control), 2) Lys provided from Smartamine ML with a targeted intake of 3 g/d metabolizable Lys (mLys; Lys-3), 3) Lys provided from Smartamine ML with a targeted intake of 6 g/d mLys (Lys-6), and 4) supplemental blood meal (AAdvantage; Perdue Agribusiness, Kings Mountain, NC) at 0.89% of dietary DM (to increase supply of mLys by 3 g/d) plus 2 g/d of metabolizable Met provided from Smartamine M (Adisseo, Alpharetta, GA; BM). The concentrations of Smartamine ML and blood meal were formulated to provide 3 or 6 g mLys/d of when intakes were 6.5 kg/d DM. However, because the diet was fed at 2.4% of BW

resulting in DM intake to range from 5.6 to 9.4 kg/d, actual intake of mLys ranged from 2.6 to 4.4 g/d for Lys-3 and BM treatment groups. For Lys-6, mLys intake ranged from 5.2 to 8.7 g/d. In addition to Lys, Smartamine ML provided Met at a 3:1 ratio of Lys to Met, so approximately 1 and 2 g/d of metabolizable Met was provided when the goal of 3 and 6 g mLys/d was provided, respectively. The four treatments were randomly assigned to each block with constraints that each treatment was replicated twice in each block but was only represented once in 4 consecutive pens that represented one half of the block. This resulted in 8 pens per treatment. Treatments were provided throughout the 77-d growing phase.

The treatment amounts of mLys were based on potential improvements in gain. Targeted improvement in gain was 10 kg over the 77-d feeding period, corresponding to an increase of 1.5 g/d in Lys deposition (130 g/d increase BW gain × 18% protein in gain × 6.4% Lys in protein; NASEM, 2016). An average efficiency value of 50% (Batista et al., 2016; Hussein et al., 2016) was used to determine the amount of mLys required to increase gain by 10 kg over 77 d. The Lys-6 treatment was twice the amount of the Lys-3 and was designed to determine if increases in performances were possible with amounts of mLys greater than those provided by Lys-3. The BM treatment provided a range of amino acids to determine if amino acids other than Lys were limiting. Additionally, BM was designed to increase the supply of mLys to the cattle by the same amount as Lys-3. AAdvantage provides 5.8 g mLys/100 g DM (Perdue AgriBusiness, 2022). Methionine was added to the BM treatment (as Smartamine M) to protect against the risk Met was limiting due to the moderate amounts of Met provided in blood meal.

The basal diet consisted of 40% wet corn gluten feed (Sweet Bran; Cargill Animal Nutrition, Blair, NE), 29.5% steam-flaked corn, 10% dry-rolled corn (mixed with treatments), 7.5% mineral and vitamin supplement, 6.5% alfalfa hay, and 6.5% prairie hay (DM basis; Table

3.2). Smartamine ML was supplemented at 0.1293% and 0.2586% of diet DM for Lys-3 and Lys-6, respectively. For Lys-3 and Lys-6, Smartamine ML was added to a small amount of dry-rolled corn (1.2% of dietary DM), mixed by hand, and added to the feed wagon as the last ingredient. The BM supplement provided all dry-rolled corn, blood meal, and Smartamine M included in the BM diet and was mixed prior to the trial in a paddle mixer and stored in an overhead bin. The corn and blood meal were mixed first, then Smartamine M was added and mixed for 1 min to evenly distribute all ingredients but prevent damage to the pH sensitive coating on the Smartamine M product. Blood meal was single-sourced from the same batch.

Cattle were fed once daily starting at 0700 h. Feed ingredients were loaded in a Roto-Mix feed wagon (Model #414-14B; Roto-Mix, Dodge City, KS). The order of ingredient additions to the feed wagon was BM supplement or dry-rolled corn, mineral and vitamin supplement, steam-flaked corn, wet corn gluten feed, alfalfa, prairie hay, and, finally, hand mixed dry-rolled corn/Smartamine ML (Lys-3 and Lys-6 diets only). Diets were mixed in the sequence of BM, control, Lys-3, Lys-6. After BM, the mixer was cleaned to prevent cross contamination of blood meal into the other diets by mixing a batch of the control treatment, which was discarded. The other three treatments were fed in increasing order of Lys content.

The mixer was cleaned after mixing Lys-6 daily to prevent cross contamination to the BM diet the next day. For cleaning, the wagon was first emptied by auger. Wet corn gluten feed, added as a flushing agent, was mixed and emptied by auger, and then the wagon was manually cleared of all accessible contents. The sequence of feeding the 4 blocks of cattle was rotated daily to balance for any differences in diet composition over the course of unloading the feed wagon. Refusals, which were rare, and amount of feed delivered to each pen was recorded daily

at feeding throughout the trial. Cattle had free access to drinking water. Samples of each feed ingredient were collected weekly and stored at -20 °C until analysis.

To verify the integrity of Smartamine products and their coating, samples of the BM supplement were collected from the paddle mixer, from the delivery truck during auguring into the overhead bin, during release from the overhead bin to the feed wagon, and from a feed bunk once delivered as total mixed ration. A sample of the hand-mixed corn and Smartamine ML mixture was also collected for analysis. Individual Smartamine beadlets were sorted from the samples and shipped to Adisseo for analysis of the coating using a ninhydrin test (Yemm et al., 1955).

Upon arrival, cattle were individually weighed (arrival weight) and grossly assessed for disease and lameness, an individual identification ear tag was applied, and each steer was ear notched for a Bovine Viral Diarrhea Virus Persistent Infection (BVD-PI) test (INDEXX SNAP BVDV Antigen Test; INDEXX Laboratories, Inc., Westbrook, ME). Two steers identified as BVDV-PI were excluded from the study prior to allocation. Steers not displaying symptoms of illness (e.g., depression, gauntness, ocular or nasal discharge) or lameness were randomly assigned to pens (~12 steers/pen) and staged until trial initiation with a consistent feeding protocol within the block. Cattle were fed 1% of BW (DM basis) of prairie hay on arrival. The next day all cattle were started on the control diet at 2.0% of BW (DM basis; dry-rolled corn, mineral and vitamin supplement, wet corn gluten feed, prairie hay, and alfalfa) until the start of the trial. Feed offerings were then increased by 0.2% of BW daily until the target 2.4% of BW was reached. If refusals were present during step-up, the leftover feed was left in the bunk and the offered feed was decreased by 2 times the amount of feed remaining. The 4 blocks were staged for 4, 4 or 9 (2 partial loads combined to form 1 block), 3, and 6 d prior to trial initiation.

Due to varying lengths of staging between blocks, pens started treatment diets at various percentages of BW. If the pen was not at the targeted 2.4% of BW (DM basis) at the time of treatment initiation, feed offerings were increased 0.2% of BW each day, if the previous day's feed was completely consumed, until the pen reached the target of 2.4% of BW (DM basis). Refusals during this period and remainder of the trial were handled the same as prior to trial initiation described above.

The trial was initiated on two separate days (2 blocks per start day) to make each day's processing more manageable. On July 10, 2020, all steers from 2 blocks were individually weighed and implanted with Revalor G (40 mg trenbolone acetate, 8 mg estradiol; Merck Animal Health, Madison, NJ). Each steer received an insecticidal ear tag (Y-TEX Warrior™ Insecticide Cattle Ear Tag with Diazinon and Chlorpyrifos; Y-Tex Corporation, Cody, WY) in both ears as well as an electronic identification (EID) ear tag. Steers were vaccinated for respiratory pathogens using Titanium 5 (Elanco Animal Health, Greenfield, IN) for IBR/BD/PI3/BRSV. Injectable Agri-mectin (Agri Laboratories Ltd., St. Joseph, MO) and Prohibit (Agri Laboratories Ltd.) drench were administered to each steer for internal and external parasites. Steers within each block were stratified to pens based on arrival weights. A metaphylactic treatment of Draxxin (tulathromycin; Zoetis, Parsippany, NJ) was injected into steers in one block because steers exhibited signs of respiratory disease. Combined body weights of all steers within a pen were measured before feeding. Steers from the final 2 blocks were started on July 13, 2020 and processed as described for the initial 2 blocks.

On d 14, before feeding, the combined weight of all steers within pens of a single block was measured before processing to minimize differences in shrink of cattle during individual steer processing. Cattle were then individually weighed and re-vaccinated with Titanium 5. A

coccygeal vein blood sample was collected from each animal into a 10-mL evacuated tube (BD Vacutainer; Beckton, Dickinson, and Company, Franklin Lakes, NJ) containing sodium heparin and immediately placed on ice. Samples were centrifuged (1,000 × g, 20 minutes, 4 °C) after sampling of each block was completed. After centrifugation, plasma was harvested into 2-mL microcentrifuge tubes and stored at -20 °C until analysis. Pen weights were subsequently measured weekly through the end of the trial. At the conclusion of the trial (d 77), cattle were also individually weighed, and coccygeal vein blood samples were collected and processed as described for d 14.

Steers were observed for clinical signs of illness twice daily, with signs including lack of interest in feed, depression, anorexia, gauntness, and ocular or nasal discharge. Animals showing signs of morbidity were moved to treatment facilities where rectal temperature and clinical illness scores were recorded. Clinical illness scores were defined as: 1: normal and healthy, 2: slightly ill, with mild depression or gauntness, 3: moderately ill, with severe depression/labored breathing/ocular or nasal discharge, or 4: severely ill, near death with little response to human approach. Steers with a clinical illness score greater than 1, a rectal temperature ≥ 40 °C, and more than 72 h post arrival were treated. After treatment, animals were returned to their pen of origin. The treatment protocol was as follows: first treatment = Nuflor (300 mg florfenicol/mL; 6 mL/45.4 kg BW subcutaneous; Merck Animal Health, Madison, NJ); second treatment = Bio-Mycin 200 (200 mg oxytetracycline/mL; 4.5 mL/45.4 kg BW subcutaneous; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO); and third treatment = Baytril 100 (100 mg enrofloxacin/mL; 6 mL/45.4 kg BW subcutaneous; Bayer Animal Health, Shawnee Mission, KS). At the third treatment, steers were considered chronically ill and removed from the trial.

The day after the last 2 blocks finished the 77-d growing phase, cattle were shipped to a commercial feedlot and mixed into 2 feedlot pens. At the feedlot, cattle did not receive the treatment diets. Due to the differing trial initiation days, the cattle were held for 1 and 4 d, receiving the control diet at 2.4% of BW, after the end of treatment before shipping. This resulted in cattle in one feedlot pen being fed for 183 or 186 d and cattle in the second feedlot pen were fed for 204 or 207 d depending on which block they were assigned during the growing phase. After the finishing period, cattle were slaughtered at a commercial facility and carcass data were acquired from the facility, including hot carcass weight, USDA Yield Grade, marbling score, ribeye area, back fat depth, and USDA Quality Grade of each carcass. Carcass data were received from the slaughter facility for 323 of the 338 (1 dead and 1 chronic were removed from study) steers that were shipped to the feedlot. Final live weights were calculated using hot carcass weight and average dressing percentage of the feedlot pen the steer occupied during the finishing period. Average daily gain (ADG) for the finishing period was calculated using the final live weight and d 77 body weight.

Sample Collection and Analysis

Each diet ingredient, excluding forages (alfalfa and prairie hay), was composited by mixing approximately 100 g obtained from each weekly sample using a sample splitter. Approximately 50 g from each weekly sample of forage were composited by separating a pile of each weekly sample into quarters and retaining two non-adjacent quarters; the process was repeated until the appropriate sample size was obtained. Samples were dried at 55 °C for 24 hours (wet corn gluten feed and BM supplement for 36 hours to ensure complete dryness) and air-equilibrated for 41 hours (29 hours for wet corn gluten feed and BM supplement). After air-equilibration, samples were weighed to determine partial DM. Alfalfa, mineral supplement,

prairie hay, and sweet bran were ground to pass a 1-mm screen using a Thomas Wiley Mill (Thomas Scientific, Swedesboro, NJ). Blood meal supplement, dry-rolled corn, and steam-flaked corn were ground to pass a 1-mm screen using a hammer mill. Ground samples were analyzed for DM by drying at 105 °C for 24 h. Samples were then placed in a muffle furnace and heated for 16 h at 450 °C to determine organic matter. Samples were analyzed for neutral detergent fiber with alpha-amylase and sodium sulfite, with residues analyzed sequentially for acid detergent fiber using an ANKOM Fiber Analyzer (Model 200, ANKOM Technology, Macedon, NY). Nitrogen content was measured using a LECO TruMac N Analyzer (LECO Corporation, Saint Joseph, MI). Crude protein was calculated as N × 6.25.

Individual blood samples were pooled by pen for analysis. Plasma urea nitrogen (PUN) was analyzed using the procedure of Marsh et al. (1965). For the analysis, 1.6 mL of PUN Color reagent and 1.2 mL of PUN acid reagent were added to 20 μL of sample. The mixtures were placed in a boiling water bath for 10 min, placed in cool water for 10 min, and analyzed using a UV/Vis Spectrometer (535 nm) within 20 min. The analysis was found to be linear up to a concentration of 6.66 mM using standards of 0, 3.33, and 6.66 mM urea.

Plasma haptoglobin was analyzed using the procedure of Cooke and Arthington (2013). Plasma haptoglobin concentrations were measured in samples pooled from all available steers within each pen at each sampling time (d 14 and 77). Only samples that showed no visual signs of hemolysis could be used, so different numbers of samples were pooled for each pen at each sampling time. Standards were prepared using a high concentration sample from a previous study that was serial diluted to create standards. In a borosilicate tube (16 × 100 mm), plasma or standard (10 μL) was added to 7.5 mL of *o*-dianisidine solution (0.6 g/L *o*-dianisidine, 0.5 g/L EDTA, and 13.8 g/L sodium phosphate monobasic in deionized water). Immediately following,

25 μL bovine hemoglobin solution (0.3 g/L bovine hemoglobin in deionized water) was added to each tube. The tubes were then covered with parafilm and incubated at 37 °C for 45 min. Directly after incubation, 100 μL of freshly prepared 156 m*M* hydrogen peroxide solution was added to each tube. The mixtures were allowed to set at room temperature for 1 h and then 200 μL from each tube was pipetted in replicates of 5 into a 96-well flat-bottom microplate. Microplates were then read using a microplate reader (450 nm; BioTek PowerWave XS, BioTek Instruments, Inc., Winooski, VT). Standards were prepared and analyzed identically to samples for every plate.

For plasma amino acids, 0.5 mL of 10% (wt/vol) sulfosalicylic acid (SSA) for deproteinization with 600 m*M* norvaline as an internal standard was added to 0.5 mL of plasma and vortexed. Samples were placed in the freezer (-20 °C) overnight to complete the deproteinization; the next day, samples were thawed, vortexed, and centrifuged at 17,000 × g at 4 °C for 15 min. The supernatant was collected and filtered through a 0.2-μm nylon syringe filter (Thermo Fisher Scientific Inc., Waltham, MA) into a vial then frozen (-20 °C) until analysis. Amino acid concentrations were analyzed by ultra-high pressure liquid chromatography (Waters Acquity UPLC; Waters Corporation, Milford, MA) using precolumn derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AccQ-Tag Ultra Derivatization Kit; Waters Corporation), separation on a C18 column, and detection with a tunable UV spectrophotometer set at 260 nm (Waters, 2007).

Statistical Analysis

Dead (1) and chronic (1) steers were removed prior to data analysis for the growing phase. Performance data from the growing phase were analyzed as a randomized block design using the mixed procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC) with a model

including the fixed effects of treatment and block. Pen was the experimental unit. Contrasts were utilized to identify linear and quadratic effects within the Lys treatments and to compare Lys-3 to BM (treatments designed to provide similar amounts of mLys).

Performance data from individual animals during the finishing phase were statistically analyzed with treatment, finishing pen, and treatment × finishing pen as fixed effects, and with block and treatment × growing-phase pen included as random effects. The random term of treatment × growing-phase pen served as the error term, so pen was ultimately treated as the experimental unit even though individual animal data were used.

Plasma urea, amino acid, and haptoglobin concentrations were statistically analyzed using the mixed procedure of SAS. Data were analyzed for a randomized block design with repeated measures (blood samples collected on d 14 and 77). Terms in the model included the fixed effect of block, treatment, sampling day, and treatment × day. Day was the repeated measure with the subject being pen. The covariance structure was compound symmetry. For haptoglobin, observations were weighted based on the number of individual samples that were pooled to make the composite samples (because samples with hemolysis could not be included). Outliers were removed when the studentized residual was > 4; 2 samples were determined to be outliers for haptoglobin (1 for Lys-3 and 1 for BM). Treatment × day effects were not significant ($P \geq 0.5$) for any plasma metabolites, so only the main effects of treatment are presented.

Statistical significance was determined at $P \leq 0.05$ and tendencies were considered $0.05 < P < 0.15$.

Results

Growing Phase Performance

Growth performance during the growing phase is presented in Table 3.3. During the growing phase (77 d) while treatments were provided, Lys-3 tended to yield greater (quadratic, $P = 0.12$) ADG compared to control. The 0.11 kg/d greater ADG resulted in approximately 9 kg of improvement in final weight at the end of the growing phase. Although not statistically different, Lys-6 led to less gain than Lys-3, resulting in about a 5 kg final weight improvement for Lys-6 compared to control. Dry matter intake was not different among groups. There was a tendency (quadratic, $P = 0.08$) for Lys-3 to have a 4% greater gain to feed ratio (G:F) than control. The BM treatment yielded results similar to control in all aspects. At d 14 and 56 of the growing phase, Lys-3 had or tended to have greater ADG compared to control and Lys-6 (d 0-14, quadratic, $P = 0.06$; d 0-56, quadratic, $P = 0.04$), as well as greater G:F (d 0-14, quadratic, $P = 0.04$; d 0-56, quadratic, $P = 0.02$).

Plasma Metabolites

Plasma urea and haptoglobin were not different between Lys groups and control ($P > 0.15$; Table 3.4). The BM treatment yielded greater ($P < 0.05$) plasma urea than Lys-3. Plasma amino acid concentrations are presented in Table 3.5. Supplemental Lys tended ($P = 0.11$) to linearly decrease plasma tyrosine concentrations but did not affect plasma concentrations of other amino acids. Compared to Lys-3, BM decreased plasma glycine concentrations ($P < 0.01$) and tended ($P = 0.11$) to decrease plasma alanine, while tending to increase lysine ($P = 0.13$) and valine ($P = 0.13$).

Finishing Phase and Carcass Performance

During the finishing phase, when steers were on feed for an average of 195 d at a commercial feedlot without receiving treatments, steers that had been fed Lys-6 during the growing phase had numerically greater (linear, $P = 0.17$) ADG than control (Table 3.6). This, in combination with slightly greater gains for Lys-6 than for control during the growing phase, resulted in 7.1 kg numerically ($P = 0.20$) heavier hot carcass weights for Lys-6 than for control. Supplementation with Smartamine ML during the growing phase linearly increased ribeye area (linear, $P = 0.05$). Steers fed Lys-3 during the growing phase had less backfat (quadratic, $P = 0.04$) by 0.19 cm compared to control and Lys-6. This contributed to steers fed Lys-3 during the growing phase having lower USDA Yield Grades (quadratic, $P = 0.02$) than steers fed control or Lys-6. Marbling scores and distribution of USDA Quality Grades were not affected by growing phase treatments.

Growth rates during the finishing phase of cattle fed BM during the growing phase were similar to those of cattle fed Lys-3 during the growing phase. Carcass characteristics were not different between cattle fed Lys-3 during the growing phase and those fed BM during the growing phase.

Discussion

Growing Phase Performance

During the growing phase, steers tended to have improved growth in response to supplementation with RP-Lys, and the benefit seemed greater for the smaller amount (Lys-3) than for the greater amount of Lys (Lys-6). The growth improvements indicate the basal diet was first limiting in lysine for our rapidly growing, limit-fed steers. Because our diets were limit-fed, gains and efficiencies were closely tied to each other. Some positive effects from RP-Lys

supplementation have been observed in light-weight cattle fed corn-based diets during the growing phase. Oke et al. (1986) observed an increase in ADG in 247-kg growing steers when provided 0.11% RP-Lys of DM while consuming 7 kg of corn-based feed (DM basis) to provide 7.7 g ruminally undegradable Lys/d; efficiency of gain was increased similarly to ADG because DMI was not affected by supplementation with RP-Lys. Williams et al. (1999) observed that ADG increased with RP-Lys supplementation, with similar results observed for 270-kg steers receiving 2.5 and 5 g mLys/d when fed a diet based on whole corn. Klemesrud et al. (2000) observed increased ADG and G:F when steers were supplemented 3 and 4 g mLys/d for the first 56 d of a 161-d trial compared to control and other amounts of Lys ranging from 1 to 12 g mLys/d. Final BW, ADG, and G:F, after 84 d of supplementation with RP-Lys at 0.4% of diet DM (11.2 g mLys/d), were greater in 184-kg Holstein steers consuming 5.6 kg/d of dietary DM (Montano et al., 2019). In comparison, Wright and Loerch (1988) observed greater growth performance in 282-kg steers fed soybean meal as the supplemental protein source than in steers fed either of 2 levels of supplemental RP-Lys as part of a corn/corn silage diet; no differences in DMI or G:F were observed among treatments. As in our study, each of these experiments included a small amount of ruminally protected Met either included in the RP-Lys product or fed to the cattle as an additional product. It is possible that the Met supplementation was responsible for the improved performance, but it is more likely due to the addition of Lys in these diets based on corn, which is starkly limiting in Lys content.

Our BM treatment group had results similar to control throughout the trial. Because the BM treatment was designed to provide amounts of mLys similar to Lys-3, this result was unexpected. Blood meal normally provides substantial amounts of Lys, but is lacking in Met (Merchen and Titgemeyer, 1992). Although we did not expect our basal diet to be limiting in

Met, we added Smartamine M, a source of ruminally protected Met, to the BM diet to ensure Met was not limiting. Cattle limit-fed a diet based on high-moisture corn at 30% of *ad libitum* intake had greater ADG when supplemented with blood meal than with soybean meal during the growing period (Loerch, 1990). Also, protein in blood meal is quite resistant to ruminal degradation (Titgemeyer et al., 1989). However, there are instances where heat processing can make amino acids, particularly Lys, unavailable for absorption due to heat damage (Waibel et al., 1977). We attempted to avoid this problem by selecting a blood meal product with high intestinal digestibility (Perdue AgriBusiness, 2022). The reason that BM, which should have provided the same amount of mLys as Lys-3, was unable to alter performance is unclear. Based on increases in plasma urea concentrations, it appears that some of the blood meal protein was absorbed, although we did not observe changes in concentrations of plasma histidine, which is present in notably high concentrations in blood meal. The lack of change in plasma histidine does not definitively indicate limitations in bioavailability of amino acids in blood meal, but it might provide limited support for that interpretation.

Plasma Metabolites

Plasma urea is responsive to changes in nitrogen intake (Hussein et al., 2016) and also can reflect changes in protein deposition (Eisemann et al., 1989), so we expected Lys supplementation that provided only negligible amount of nitrogen would reduce plasma urea concentrations if it was a limiting amino acid that increased protein deposition. In 165-kg Holstein steers plasma urea tended to linearly decrease with supplemental Lys (Batista et al., 2016). The BM treatment likely led to greater plasma urea concentrations when compared to Lys-3 because intake of dietary nitrogen was greater.

Haptoglobin reflects inflammation status in cattle, therefore, reflecting the general health and stress level of the animals (Godson et al., 1996). We did not observe treatment effects for plasma haptoglobin, suggesting that our supplements did not affect systemic inflammation. At the same time, our steers were purchased and transported in a low-stress manner, which might limit expression of differences among treatments.

With increasing Lys supply, plasma Lys might be expected to increase, and previous work (Hussein et al., 2016) has demonstrated increases in plasma Lys concentration in response to supplemental Lys. Increases in plasma Lys in response to supplementation could indicate the requirement had been exceeded (Williams and Smith, 1974), although the increases in plasma Lys observed by Hussein et al. (2016) occurred when Lys supplies were less than the requirement. Bergen (1979) suggested that supplementation of amino acids in amounts below the requirement would increase protein deposition and therefore not lead to increases in plasma concentrations. In growing steers (247 kg) fed a corn/corn-silage-based diet, RP-Lys supplementation increased gains but did not lead to significant increases in plasma lysine concentration (Oke et al., 1986). As well, Batista et al. (2016) observed 3 g/d of abomasally infused Lys minimally increased plasma Lys over control, but plasma Lys concentration was increased with additional Lys supply between 6 to 12 g/d supplemental Lys; in that study, the requirement for maximal nitrogen retention was 9 g/d supplemental Lys. Similarly, plasma Lys was unaffected by abomasal infusion of 4 g/d Lys, but linearly increased with 8 and 12 g/d infused Lys (Titgemeyer et al., 1988). The tendency for increased ADG and greater final weight in the growing phase indicates the Lys supplemented steers in our study were utilizing at least some of the supplemental Lys for protein deposition, which likely led to the similar plasma Lys concentrations among treatments.

Finishing Phase and Carcass Performance

Steers did not receive treatments during the finishing phase at the commercial feedlot. The Lys-6 treatment was numerically more effective in enhancing growth performance during the finishing phase, although it was somewhat less effective than Lys-3 during the growing phase when the treatments were applied. One possible explanation is that Lys-6 led to greater intakes during the finishing phase when limit-feeding ceased. However, this cannot be confirmed because treatment groups were commingled in pens during finishing such that treatment differences in DMI could not be measured. During the finishing phase, the Lys-3 treatment did not lose the advantage in BW achieved during the growing phase. Even with ADG similar to the control group during the finishing phase, Lys-3 led to carcass weights numerically heavier than control with ribeye area greater than control and similar those of the somewhat heavier carcasses of steers fed Lys-6. Zinn et al. (2007) compared growth performance and carcass characteristics in Holstein steers (114 kg) fed a diet based on steam-flaked corn. Diets were formulated to meet average metabolizable amino acid requirements either for the total 351-d trial (control) or for the initial 112 d phase followed by cattle being fed the control diet for the remainder of the trial. During the first 112 d, diets were formulated for metabolizable amino acid requirements either for the whole 112 d or for two 56-d periods, but these treatment groups did not differ in results. The control diet provided only 70-88% of the calculated requirements for Lys, Met, and histidine during first 112 d. When steers were provided adequate amounts of amino acids during the initial growing period with the phased diets, ADG, DMI, and G:F were increased compared to control during this time period. Performance was not different between treatment groups from d 112 to 351. However, the impacts on growth were maintained through the entirety of the trial with ADG, DMI, and G:F being greater in phased cattle over the total 351-d trial. This resulted in 5%

larger hot carcass weights with 25% more fat thickness and 10% larger ribeye area for the phased cattle. This response is similar to our Lys-3 treatment and indicates benefits of supplementing growing steers during the initial phases of feeding can be maintained during the latter phases of growth.

Results like ours have been observed in previous studies, but where RP-Lys was supplemented during the finishing phase. Hussein and Berger (1995) observed overall ADG was greater than control with supplementation providing 2.5 and 5 g mLys/d fed during the 266-d trial, resulting in a greater final BW. Oke et al. (1986) observed numerically greater ADG (by 8%) and hot carcass weights (by 6%) in steers (368 kg) supplemented with 5 g ruminally undegradable Lys/d during the finishing period compared to control. Steers supplemented 3 and 4 g mLys/d had greater ADG when compared to the control group (Klemesrud et al., 2000). In a similar manner to Oke et al., (1986), this resulted in numerically larger hot carcass weights of 22 kg in cattle receiving these treatments over the control. These improvements in performance suggest supplemental Lys in the growing phase may impact finishing performance and the resulting carcass of cattle fed corn-based diets.

Conclusion

When steers were fed a corn-based diet, RP-Lys supplementation provided in the growing phase improved growth performance of cattle. The lower amount of RP-Lys had a greater effect during the growing phase. However, after treatments were terminated and cattle moved to the finishing phase, the larger amount of RP-Lys had a greater effect on growth, resulting in heavier carcass weights. Supplementation of blood meal during the growing phase did not affect growth performance in either the growing or finishing phases, despite the expectation that it provided as much mLys as the lower RP-Lys treatment.

Literature Cited

Batista, E. D., A. H. Hussein, E. Detmann, M. D. Miesner, and E. C. Titgemeyer. 2016. Efficiency of lysine utilization by growing steers. J. Anim. Sci. 94:648–655. doi:10.2527/jas.2015-9716.

Bergen, W. G. 1979. Free amino acids in blood of ruminants--physiological and nutritional regulation. J. Anim. Sci. 49:1577–1589. doi:10.2527/jas1979.4961577x.

Cooke, R. F., and J. D. Arthington. 2013. Concentrations of haptoglobin in bovine plasma determined by ELISA or a colorimetric method based on peroxidase activity. Journal of Animal Physiology and Animal Nutrition. 97:531–536. doi:10.1111/j.1439-0396.2012.01298.x.

Cottle, D. J., and W. Velle. 1989. Degradation and outflow of amino acids from the rumen of sheep. Br. J. Nutr. 61:397–408. doi:10.1079/BJN19890126.

Eisemann, J. H., A. C. Hammond, T. S. Rumsey, and D. E. Bauman. 1989. Nitrogen and protein metabolism and metabolites in plasma and urine of beef steers treated with somatotropin. J. Anim. Sci. 67:105–115. doi:10.2527/jas1989.671105x.

Godson, D. L., M. Campos, S. K. Attah-Poku, M. J. Redmond, D. M. Cordeiro, M. S. Sethi, R. J. Harland, and L. A. Babiuk. 1996. Serum haptoglobin as an indicator of the acute phase response in bovine respiratory disease. Vet. Immunol. Immunopathol. 51:277–292. doi:10.1016/0165-2427(95)05520-7.

Hussein, A. H., E. D. Batista, M. D. Miesner, and E. C. Titgemeyer. 2016. Effect of ruminal ammonia supply on lysine utilization by growing steers. J. Anim. Sci. 94:656–664. doi:10.2527/jas.2015-9717.

Hussein, H. S., and L. L. Berger. 1995. Feedlot performance and carcass characteristics of Holstein steers as affected by source of dietary protein and level of ruminally protected lysine and methionine. J. Anim. Sci. 73:3503–3509. doi:10.2527/1995.73123503x.

Klemesrud, M. J., T. J. Klopfenstein, R. A. Stock, A. J. Lewis, and D. W. Herold. 2000. Effect of dietary concentration of metabolizable lysine on finishing cattle performance. J. Anim. Sci. 78:1060–1066. doi:10.2527/2000.7841060x.

Loerch, S. C. 1990. Effects of feeding growing cattle high-concentrate diets at a restricted intake on feedlot performance. J. Anim. Sci. 68:3086–3095. doi:10.2527/1990.68103086x.

Marsh, W. H., B. Fingerhut, and H. Miller. 1965. Automated and manual direct methods for the determination of blood urea. Clin. Chem. 11:624–627. doi:10.1093/clinchem/11.6.624.

Merchen, N. R., and E. C. Titgemeyer. 1992. Manipulation of amino acid supply to the growing ruminant. J. Anim. Sci. 70:3238–3247. doi:10.2527/1992.70103238x.

Montano, M. F., J. O. Chirino, B. C. Latack, J. Salinas-Chavira, and R. A. Zinn. 2019. Influence of supplementation of growing diets enriched with rumen-protected methionine and lysine on feedlot performance and characteristics of digestion in Holstein steer calves. Appl. Anim. Sci. 35:318–324. doi:10.15232/aas.2019-01843.

NASEM. 2016. Nutrient requirements of beef cattle. 8th ed. Natl. Acad. Press, Washington, DC.

Oke, B. O., S. C. Loerch, and L. E. Deetz. 1986. Effects of rumen-protected methionine and lysine on ruminant performance and nutrient metabolism. J. Anim. Sci. 62:1101–1112. doi:10.2527/jas1986.6241101x.

Perdue AgriBusiness. 2022. Aadvantage Specifications. Available from: https://www.perdueagribusiness.com/agribusiness/media/1078/aadvantage.pdf

Richardson, C. R., and E. E. Hatfield. 1978. The limiting amino acids in growing cattle. J. Anim. Sci. 46:740–745. doi:10.2527/jas1978.463740x.

Titgemeyer, E. C., N. R. Merchen, and L. L. Berger. 1988. Estimation of lysine and methionine requirements of growing steers fed corn silage-based or corn-based diets. J. Dairy Sci. 71:421–434. doi:10.3168/jds.S0022-0302(88)79572-7.

Titgemeyer, E. C., N. R. Merchen, and L. L. Berger. 1989. Evaluation of soybean meal, corn gluten meal, blood meal and fish meal as sources of nitrogen and amino acids disappearing from the small intestine of steers. J. Anim. Sci. 67:262–275. doi:10.2527/jas1989.671262x.

Waibel, P. E., M. Cuperlovic, R. F. Hurrell, and K. J. Carpenter. 1977. Processing damage to lysine and other amino acids in the manufacture of blood meal. J. Agric. Food Chem. 25:171–175. doi:10.1021/jf60209a035.

Waters. 2007. UPLC Amino Acid Analysis Solution. System Guide. Waters Corporation, Milford, MA. Available from: https://waters.com/webassets/cms/support/docs/71500129702rb.pdf

Williams, A. P., and R. H. Smith. 1974. Concentrations of amino acids and urea in the plasma of the ruminating calf and estimation of the amino acid requirements. Br. J. Nutr. 32:421–433. doi:10.1079/BJN19740094.

Williams, J. E., S. A. Newell, B. W. Hess, and E. Scholljegerdes. 1999. Influence of rumen-protected methionine and lysine on growing cattle fed forage and corn based diets. J. Prod. Agric. 12:696–701. doi:10.2134/jpa1999.0696.

Wright, M. D., and S. C. Loerch. 1988. Effects of rumen-protected amino acids on ruminant nitrogen balance, plasma amino acid concentrations and performance. J. Anim. Sci. 66:2014–2027. doi:10.2527/jas1988.6682014x.

Yemm, E. W., E. C. Cocking, and R. E. Ricketts. 1955. The determination of amino-acids with ninhydrin. Analyst. 80:209–214. doi:10.1039/AN9558000209.

Zinn, R. A., J. F. Calderón, L. Corona, and A. Plascencia. 2007. Phase feeding strategies to meet metabolizable amino acid requirements of calf-fed Holstein steers. Prof. Anim. Sci. 23:333–339. doi:10.15232/S1080-7446(15)30986-4.

Table 3.1 Composition of treatment diets fed to steers during growing phase

	Treatment[1]			
Ingredient, % of dry matter	Control	Lys-3	Lys-6	BM
Smartamine ML[2]	0	0.13	0.26	0
Smartamine M[2]	0	0	0	0.05
Blood meal[3]	0	0	0	0.89
Dry-rolled corn	10.0	9.9	9.7	9.1
Steam-flaked corn	29.5	29.5	29.5	29.5
Wet corn gluten feed[4]	40.0	40.0	40.0	40.0
Alfalfa hay	6.5	6.5	6.5	6.5
Prairie hay	6.5	6.5	6.5	6.5
Supplement[5]	7.5	7.5	7.5	7.5

[1] Control = no supplemental amino acids/protein, Lys-3 = 3 g/d metabolizable Lys from Smartamine ML, Lys-6 = 6 g/d metabolizable Lys from Smartamine ML, BM = supplemental blood meal at 0.89% of dietary dry matter plus 2 g/d of metabolizable Met provided from Smartamine M.

[2] Adisseo, Alpharetta, GA.

[3] Aadvantage; Perdue Agribusiness, Kings Mountain, NC.

[4] Sweet Bran, Cargill Animal Nutrition, Blair, NE.

[5] Supplement pellet formulated to contain (DM basis) 8.4% Ca, 5.0% NaCl, and 360 mg/kg monensin. Supplement ingredients (DM basis): 72.15% wheat middlings, 22.0% calcium carbonate, 5.0% NaCl, 0.35% soybean oil, 0.18% Rumensin 90 (Elanco), 0.11% $ZnSO_4$, 0.08% $MnSO_4$ (32% Mn), 0.06% vitamin E premix (500,000 IU/kg), 0.05% $CuSO_4$, 0.01% Se premix

(0.99% Se), 0.007% ethylenediamine dihydriodide premix (11.4% EDDI), 0.004% vitamin A premix (650,000 IU/g).

Table 3.2. Nutrient composition of ingredients and basal diet fed during the growing phase

	Nutrient[1]				
Item	DM	OM	CP	NDF	ADF
Ingredient, % of dry matter					
Blood meal supplement[2]	86.3	98.1	15.0	22.6	3.0
Dry-rolled corn	84.8	98.6	8.6	16.0	2.7
Steam-flaked corn	81.0	98.4	8.5	28.8	3.1
Wet corn gluten feed	58.3	93.7	24.4	40.9	10.0
Alfalfa hay	86.6	88.2	21.6	49.7	30.8
Prairie hay	89.3	92.0	5.0	70.8	37.2
Supplement	92.9	67.2	11.7	31.9	10.6
Control diet[3]	74.1	93.1	15.8	36.7	10.4
Blood meal diet[3]	74.3	93.1	16.4	37.3	10.4

[1] DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber.

[2] BM supplement contained 90.6% dry-rolled corn, 8.9% blood meal and 0.513% Smartamine M (DM basis), and it was used in BM treatment diet in replacement of dry-rolled corn.

[3] Based on analysis of individual feed ingredient samples.

Table 3.3. Growth performance of limit-fed steers supplemented ruminally protected lysine or blood meal during the growing phase

	Treatment[1]					Contrast *P*-value[2]		
Item	Control	Lys-3	Lys-6	BM	SEM[3]	Lys-Linear	Lys-Quad	Lys-3 vs. BM
No. of pens	8	8	8	8				
No. of steers	85	82	86	85				
Body weight, kg								
d 0	249.1	247.9	248.6	248.7	1.45	0.83	0.60	0.68
d 7	273.0	271.3	272.2	207.4	2.33	0.81	0.64	0.78
d 14	286.9	288.6	286.7	284.6	2.00	0.95	0.45	0.16
d 56	358.9	363.7	358.4	354.0	2.90	0.90	0.17	0.03
d 77	393.7	401.3	397.9	392.8	3.87	0.45	0.26	0.13
Average daily gain, kg/d								
d 0 to 7	3.42	3.34	3.37	3.09	0.255	0.89	0.86	0.49
d 0 to 14	2.70	2.91	2.72	2.56	0.083	0.88	0.06	0.01
d 0 to 56	1.96	2.07	1.96	1.88	0.041	0.98	0.04	<0.01
d 0 to 77	1.88	1.99	1.94	1.87	0.042	0.32	0.12	0.05
Dry matter intake, kg/d								
d 0 to 7	5.64	5.65	5.74	5.80	0.090	0.41	0.69	0.25
d 0 to 14	6.07	6.05	6.10	6.11	0.064	0.73	0.64	0.50
d 0 to 56	7.19	7.23	7.20	7.15	0.057	0.89	0.61	0.33
d 0 to 77	7.66	7.73	7.68	7.63	0.061	0.77	0.41	0.23

Gain:feed, kg/kg								
d 0 to 7	0.644	0.623	0.599	0.543	0.0526	0.55	0.98	0.29
d 0 to 14	0.453	0.489	0.451	0.424	0.0137	0.90	0.04	<0.01
d 0 to 56	0.275	0.288	0.274	0.265	0.0043	0.94	0.02	<0.01
d 0 to 77	0.247	0.259	0.254	0.247	0.0040	0.25	0.08	0.04

[1] Control = no supplemental amino acids/protein, Lys-3 = 3 g/d metabolizable Lys from Smartamine ML, Lys-6 = 6 g/d metabolizable Lys from Smartamine ML, BM = supplemental blood meal at 0.89% of dietary dry matter plus 2 g/d of metabolizable Met provided from Smartamine M.

[2] Lys-Linear = control vs. Lys-6, Lys-Quad = Lys-3 vs. average of control and Lys-6.

[3] Average standard error of mean among treatments.

Table 3.4. Plasma concentrations of limit-fed steers supplemented ruminally protected lysine or blood meal during the growing phase collected on days 14 and 77

	Treatment[1]					Contrast *P*-value[2]		
Item	Control	Lys-3	Lys-6	BM	SEM[3]	Lys-Linear	Lys-Quad	Lys-3 vs. BM
No. of pens	8	8	8	8				
Urea nitrogen, mM	4.61	4.61	4.70	4.94	0.087	0.43	0.69	0.01
Haptoglobin, mg/L	1324	1559	1543	1315	110	0.16	0.37	0.13

[1] Control = no supplemental amino acids/protein, Lys-3 = 3 g/d metabolizable Lys from Smartamine ML, Lys-6 = 6 g/d metabolizable Lys from Smartamine ML, BM = supplemental blood meal at 0.89% of dietary dry matter plus 2 g/d of metabolizable Met provided from Smartamine M.

[2] Lys-Linear = control vs. Lys-6, Lys-Quad = Lys-3 vs. average of control and Lys-6

[3] Average standard error of mean among treatments.

Table 3.5. Plasma amino acid concentrations of limit-fed steers supplemented ruminally protected lysine or blood meal during the growing phase

Amino acid, μM	Treatment[1]					Contrast *P*-value[2]		
	Control	Lys-3	Lys-6	BM	SEM[3]	Lys-Linear	Lys-Quad	Lys-3 vs. BM
No. of pens	8	8	8	8				
Alanine	233.11	239.00	232.08	226.29	5.471	0.89	0.35	0.11
Arginine	148.34	150.09	153.78	148.75	3.360	0.26	0.81	0.78
Asparagine	62.32	61.02	62.60	63.39	1.251	0.87	0.35	0.20
Aspartate	14.35	14.30	13.91	13.80	0.328	0.34	0.68	0.29
Glutamate	264.45	262.86	265.66	259.21	3.507	0.81	0.61	0.47
Glutamine	104.23	104.41	108.79	99.34	3.074	0.30	0.58	0.26
Glycine	340.37	338.89	344.86	303.77	7.420	0.67	0.68	0.003
Histidine	28.67	27.75	28.37	26.93	0.761	0.78	0.41	0.46
Isoleucine	107.14	107.91	107.81	105.13	2.325	0.84	0.88	0.41
Leucine	180.61	181.92	183.31	189.99	4.023	0.64	0.99	0.17
Lysine	105.06	101.14	104.66	107.44	2.856	0.92	0.29	0.13
Methionine	34.56	34.59	36.00	36.03	1.038	0.33	0.59	0.34
Ornithine	78.80	76.51	79.54	80.51	1.983	0.79	0.28	0.17
Phenylalanine	58.20	58.86	58.01	59.04	1.383	0.93	0.66	0.93
Proline	80.92	80.72	82.88	79.64	2.115	0.52	0.65	0.72
Serine	44.33	47.49	46.32	48.85	1.387	0.32	0.21	0.50
Taurine	37.37	38.07	37.81	36.23	1.818	0.80	0.74	0.29

Threonine	73.20	74.19	72.94	73.19	2.016	0.93	0.66	0.73
Tryptophan	43.48	45.03	45.13	43.49	1.251	0.36	0.64	0.40
Tyrosine	69.29	67.54	65.28	64.65	1.749	0.11	0.91	0.26
Valine	254.46	264.69	263.54	278.44	6.108	0.30	0.45	0.13
Total	2363.29	2376.97	2393.27	2344.42	32.566	0.52	0.97	0.49

[1] Control = no supplemental amino acids/protein, Lys-3 = 3 g/d metabolizable Lys from Smartamine ML, Lys-6 = 6 g/d metabolizable Lys from Smartamine ML, BM = supplemental blood meal at 0.89% of dietary dry matter plus 2 g/d of metabolizable Met provided from Smartamine M.

[2] Lys-Linear = control vs. Lys-6, Lys-Quad = Lys-3 vs. average of control and Lys-6

[3] Average standard error of mean among treatments.

Table 3.6. Finishing phase growth performance and carcass characteristics of steers supplemented ruminally protected lysine or blood meal during the growing phase

	Treatment[1]					Contrast *P*-value[2]		
Item	Control	Lys-3	Lys-6	BM	SEM[3]	Lys-Linear	Lys-Quad	Lys-3 vs. BM
No. of steers	81	79	82	81				
Day 0 body weight, kg	264.0	262.3	261.2	260.6	13.7	0.49	0.94	0.69
Day 14 body weight, kg	295.7	296.1	294.2	293.9	12.5	0.70	0.75	0.59
Day 77 body weight, kg	406.2	411.9	407.1	403.7	10.4	0.85	0.20	0.09
Slaughter body weight, kg	672.8	678.1	683.8	672.1	5.9	0.20	0.98	0.48
Finishing daily gain, kg/d	1.37	1.37	1.42	1.38	0.059	0.17	0.39	0.80
Hot carcass weight, kg	434.2	437.6	441.3	433.7	3.8	0.20	0.99	0.48
USDA Yield Grade	3.15	2.77	2.92	2.95	0.14	0.08	0.02	0.17
Marbling score[4]	578	554	557	562	14.8	0.25	0.40	0.66
Ribeye area, cm^2	94.7	97.7	97.5	96.0	1.6	0.05	0.18	0.21
Backfat, cm	1.87	1.68	1.80	1.79	0.060	0.36	0.04	0.19

USDA Prime + Choice, %	98.3	97.1	99.2	95.5	2.3	0.75	0.53	0.57
USDA Prime, %	11.1	8.4	5.5	14.7	3.9	0.28	0.98	0.24
USDA Choice, %	87.1	88.7	93.7	90.7	4.3	0.26	0.74	0.18
USDA Select, %	1.7	2.9	0.8	4.5	2.3	0.75	0.53	0.57

[1] Treatments provided during the 77-day growing phase, but not during the finishing phase. Control = no supplemental amino acids/protein, Lys-3 = 3 g/d metabolizable Lys from Smartamine ML, Lys-6 = 6 g/d metabolizable Lys from Smartamine ML, BM = supplemental blood meal at 0.89% of dietary dry matter plus 2 g/d of metabolizable Met provided from Smartamine M.

[2] Lys-Linear = control vs. Lys-6, Lys-Quad = Lys-3 vs. average of control and Lys-6

[3] Average standard error of mean among treatments.

[4] 500 = Modest^{00}; 600 = Moderate^{00}

Chapter 4 - Effect of guanidinoacetic acid supplementation on performance of milk-fed calves

Kathryn J. Hazlewood, Charles A. Zumbaugh, Cassandra A. Jones, Emily M. Atkinson, Hannah L. R. Tingler, Vivienne K. Inhuber, Micheal J. Brouk, and Evan C. Titgemeyer

Abstract

Guanidinoacetic acid (GAA) is the direct precursor to creatine, which serves as an energy reserve mechanism in the body. Supplementation of GAA can increase creatine synthesis and promote growth performance of animals. Our objective was to evaluate the effect of GAA supplementation on growth performance of milk-fed calves and subsequent growth performance during the weaning period. Forty-five (5- to 9-d old) Holstein-Angus steer calves (40.9 ± 3.6 kg) were blocked by arrival BW and total serum protein and assigned to 1 of 3 treatments to assess growth performance. Treatments were provided in the milk replacer by bottle for 42 d and included 0, 1, or 2 g/d GAA (Creamino; Alzchem Trostberg GmbH, Trostberg, Germany). Daily treatments were divided into 2 feedings. Calves were housed individually and fed 2.84 L milk replacer (25% protein, 24% fat) twice daily. For the entirety of the trial, *ad libitum* access to starter feed and water was provided. No treatment was fed during the 17-d weaning period. During weaning, calves received 1.89 L of milk replacer twice daily from d 42 to 48, 1.89 L of milk replacer once daily from d 49 to 55, and no milk replacer after d 55. Health was assessed and scored twice daily for respiratory disease, fecal consistency, and lameness during treatment provision and once daily during the weaning phase. Body weight and hip height were measured on d 0, 14, 28, and 42. Body weight was also measured on day 59 (weaning weight). Due to death loss and removal of 1 calf, performance data of 41 calves were analyzed. There was a tendency for BW to linearly increase ($P = 0.09$) with increasing GAA by d 59. Hip height increased with increasing GAA on d 14 (linear, $P = 0.005$). With GAA supplementation, average daily gain (ADG) tended to increase from d 0 to 42 (linear, $P = 0.15$) and d 42 to 59 (linear, $P = 0.14$). Overall, ADG tended to increase with increasing GAA (linear, $P = 0.09$). During all time periods, starter feed dry matter intake tended to linearly increase ($P \leq 0.07$) with GAA

supplementation. The 2 levels of GAA supplementation yielded similar results during the trial. No significant differences among treatments ($P > 0.05$) were observed in health scores during treatment or weaning phases. Inclusion of GAA in milk replacer tended to increase gain of calves, and this was associated with elevated starter feed intake.

Key words: cattle, guanidinoacetic acid, milk-fed.

Introduction

Guanidinoacetic acid (GAA) is the direct precursor to creatine (Brosnan et al., 2009). Creatine plays a major role in energy metabolism of the body as a reserve during times of high-intensity work. Phosphorylated creatine (phosphocreatine) is a high energy substrate that is used for fast regeneration of adenosine triphosphate (ATP) by phosphorylating adenosine diphosphate (ADP), a reaction catalyzed by creatine kinase. Synthesis of creatine requires 3 amino acids in a 2-step process beginning with the amidino group from Arg transferred to Gly to generate GAA. This reaction is catalyzed by L-arginine:glycine amidinotransferase (AGAT) and mainly occurs in the kidney. Production of GAA is repressed by high creatine concentrations (Wyss and Kaddurah-Daouk, 2000). The GAA is methylated at the amidino group by S-adenosyl-L-methionine:N-guanidinoacetate methyltransferase (GMAT) in the liver resulting in creatine (Wyss and Kaddurah-Daouk, 2000). Creatine is then released into the circulatory system for uptake via the creatine transporter into creatine containing cells, predominantly skeletal and heart muscle (Brosnan and Brosnan, 2007).

Young growing animals require creatine to support tissue growth (Brosnan et al., 2009). Creatine supplementation is widely used by humans (Brosnan and Brosnan, 2007) and has been studied in many animal experiments. However, high serum creatine concentrations repress expression and activity of AGAT and the creatine transporter (Wyss and Kaddurah-Daouk, 2000). Thus, creatine supplementation can result in less production of GAA which therefore leads to less endogenous synthesis of creatine. In turn, muscle creatine concentrations may be only minorly affected, even when serum creatine is elevated, due to inhibition of transport into the cell (Wyss and Kaddurah-Daouk, 2000).

Supplementation of GAA, in comparison, appears to increase creatine concentrations in serum and muscle more effectively than creatine supplementation (Ostojic et al., 2016). Studies in poultry and swine have yielded positive results in growth performance, meat quality, and antioxidant stress (Michiels et al., 2012; Wang et al., 2012; He et al., 2018; Jayaraman et al., 2018) in response to GAA supplementation. Use of GAA is approved by both the European Food Safety Authority (EFSA) and United States Food and Drug Administration (FDA) for poultry and by the EFSA for swine (EFSA, 2009; FDA, 2021). Conversely, it is not currently approved for use in cattle. Some work is available on the metabolism of GAA and supplementation to growing bulls and lambs, but there is no information available in milk-fed calves. Our objective was to evaluate the effect of GAA supplementation on growth performance of milk-fed calves and the subsequent growth performance during the weaning period.

Materials and Methods

All procedures involving the use of animals were approved by the Kansas State University Institutional Animal Care and Use Committee.

Animals and Experimental Diets

A 42-d growth study was conducted using 49 Holstein-Angus cross steer calves of 5 to 9 d of age at initiation of the trial (40.9 ± 3.55 kg initial body weight; BW). Calves were obtained in one load from a calf ranch (Fullmer Calf Ranch, Syracuse, KS). Prior to arrival, calves received colostrum on farm of birth, were transported to the calf ranch at 1 to 2 d of age, were fed milk replacer for 1 to 4 d, and then were transported to Manhattan, KS 3 d before the trial was initiated. Calves were weighed on arrival then individually housed in hutches (Calf-tel, Germantown, WI) and bedded with a layer of pine wood shavings with straw added heavily on top. Calves were staged for 3 d until the initiation of the trial, during which they were fed milk replacer and introduced to starter feed, both being the same fed during the trial (Table 4.1). A health assessment was conducted by the Kansas State Veterinary Health Center, and all calves received a subcutaneous dose of florfenicol (40 mg/100 kg BW; Nuflor; Merck Animal Health, Madison, NJ) on d -1 or -2.

A jugular vein blood sample was collected on d -1 into a 3-mL vacutainer tube without anticoagulant (BD Vacutainer; Beckton, Dickinson, and Company, Franklin Lakes, NJ) at 1 to 3 h after feeding. Blood was allowed to coagulate and then centrifuged at 1,000 × *g* at 4 °C for 20 min. Serum was harvested and total serum protein was measured using refractometry (Deelen et al., 2014) as an assessment of passive transfer from colostrum. Deelen et al. (2014) showed refractometry measurements correlated well with serum IgG concentrations. For allocation to treatment, calves were grouped into uniform blocks of 3 animals based on arrival BW and total

serum protein to achieve groups of calves with similar weight and total serum protein concentrations. Treatments included 0, 1, or 2 g/d of Creamino (96% GAA; Alzchem Trostberg GmbH, Trostberg, Germany). Only 45 calves were blocked and allocated to treatment due to death loss and inconsistent intake of milk replacer. One calf from each block was randomly assigned to each treatment group, resulting in 15 animals per group. One calf supplemented 2 g GAA/d died within 24 h of trial initiation and was replaced by one of the extra calves not initially allotted to the trial.

Calves were fed 2.84 L milk replacer (0.124 g dry matter/mL, 0.156 g dry matter/g) twice daily at 0700 and 1800 h, with each calf using the same bottle and nipple through the entirety of the trial. Milk replacer (K-State 26/24 AM DX; Milk Specialties Global, Eden Prairie, MN; Table 4.1) with decoquinate (26.69 mg/kg) and 0.5 g/calf probiotic (Certillus Calf LC; Church & Dwight Co., Inc, Waukesha, WI) was added to warm water (43.3 °C) and mixed for 4 to 5 min for each batch. Two batches of equal amounts were used to make enough milk replacer for all calves at each feeding. Half of the daily GAA treatment amount was added directly to each bottle before the nipple was placed on the bottle and shaken. The treatment product was added in its original dry form through d 10. At this point it was determined the product was not fully suspended in the milk replacer, which resulted in some unconsumed product in the bottles after feeding. From d 11 to 42, for the 2 g GAA/d treatment, 1 g of product was mixed with 2 mL of water and 1 mL 4.5 M HCl, allowed to set for 20 min, and then neutralized with 1 mL 18% wt/wt NaOH prior to addition into the bottles; for the 1 g GAA/d treatment, mixtures used one-half the amounts described. Milk replacer was fed to calves between 37.8 and 40.6 °C. Calves were fed in the same sequence at each feeding. Milk replacer refusals were measured after each feeding.

Starter feed (Elite 18% Calf Starter TRT D22.7; Hubbard Feeds, Mankato, MN) medicated with decoquinate (50.04 mg/kg) was fed to calves individually for *ad libitum* intake through the entirety of the study (Table 4.1). Starter was provided at the morning feeding and refusals were measured the following morning to calculate daily intake. Initially, calves received 0.045 kg that was replaced daily for the first 10 d. After this point, a set amount of feed was determined every morning with the goal to maintain *ad libitum* access. Refusals were measured and, if leftover feed was dry and uncontaminated, new feed was added to the refusals until the set amount was reached. If feed was wet to the touch, all feed was discarded; a sample was composited from the discarded feed and analyzed for dry matter (DM). If little or no feed was left at the evening feeding, the full daily amount of feed was added. For instances where feed refusals were inadvertently not weighed, the daily starter intake was calculated as the average from the previous and following daily intakes; this constituted 2.23% of all observations. Water was provided for *ad libitum* access and refreshed at each feeding.

Bottles and nipples were cleaned after each feeding. Bottles were first washed with warm water and soap and then fully submerged in a 0.016% chlorohexidine solution (Chlorhexidine Solution; Aspen Vet, Loveland, CO) for 20 to 30 sec and air-dried. Due to health concerns, a third step was added on d 26 with bottles briefly submerged in a 0.6% wt/vol bleach solution and air-dried. Nipples were cleaned using the same process. The milk replacer mixer was cleaned and sanitized after each feeding. It was first emptied of any remaining milk replacer, rinsed with hot water until clear, and filled with 0.016% chlorohexidine solution, which was retained for 1 to 2 min. The chlorohexidine solution was drained from the mixer and the lid was removed until air-dry. Manure and wet straw were removed from hutches daily in the evening. After the first 2 weeks and weekly thereafter, hutches were completely cleaned out and new straw was added.

Health was assessed twice daily at each feeding. An evaluation of each animal was based on a scale of 0-3 for respiratory, lameness, and fecal consistency using the following system adapted from the University of Wisconsin-Madison School of Veterinary Medicine Calf Health Scoring Chart (chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/viewer.html?pdfurl=https%3A%2F%2Ffyi.extension.wisc.edu%2Fheifermgmt%2Ffiles%2F2015%2F02%2Fcalf_health_scoring_chart.pdf&clen=270894; McGuirk and Peek, 2014). Evaluation of respiratory health was the sum of general appearance, eye and nasal discharge, and cough scores. Adaptations included the addition of a general appearance category with 0 = energetic, normal demeanor, and comes to feed eagerly, 1 = slightly depressed and does not come to feed as readily, 2 = depressed demeanor, low energy, and does not come to feed, and 3 = depressed demeanor and sickly looking. Cough was scored as yes = 1 or no = 0. Rectal temperature and ear tilt score was omitted. If respiratory health score was greater than or equal to 4, a veterinarian was consulted. If prescribed by the veterinarian, florfenicol (40 mg/100 kg BW; Nuflor; Merck Animal Health, Madison, NJ) was injected subcutaneously and that animal was observed carefully for further respiratory symptoms; no second treatments were needed. For fecal consistency, when the animal scored a 2 and had low milk replacer intake or scored a 3, a half dose (1 dose = 1.89 L) of electrolyte solution was fed between feedings. One half to one full dose of electrolyte were fed at the time of feeding if the calf refused all milk replacer. Calves received one half to one dose of electrolyte, according to severity, until 1 d after fecal consistency and intake improved. Electrolyte was first attempted to be fed by bottle and, if refused, was tubed. Epic (Tomlyn Products, Fort Worth, TX) was used from d 0 to 1, Re-sorb (Zoetis, Kalamazoo, MI) was used from d 2 to d 21, and Hydra-Lyte

(AgriLabs, Shenandoah, IA) was used from d 21 to d 38, based on availability. No doses of electrolyte were needed after d 38.

Due to extreme dehydration from scouring, a calf receiving 2 g GAA /d was taken to the Kansas State University Veterinary Health Center for care after the morning feeding on d 4. Fluids with 1.3% sodium bicarbonate were given intravenously for the first day and then transitioned to isotonic fluids for the following day. While there, the calf was fed 0.5 L of milk replacer without treatment every 4 h and then transitioned to 1.5 L every 6 h. Three doses of Excenel RTU EZ (Ceftiofur Hydrochloride; Zoetis, Kalamazoo, MI) were administered subcutaneously every 24 h. After 2 d of care, the calf was returned to the experiment on d 6.

Body weight and hip height were measured on d 0, 14, 28, and 42 for assessment of growth performance during treatment provision. On d 14, 28, and 42, a jugular vein blood sample was collected into a 10-mL vacutainer tube (BD Vacutainer; Beckton, Dickinson, and Company, Franklin Lakes, NJ) containing sodium heparin starting 3.5 h after feeding. Tubes were immediately inverted multiple times and placed in ice until centrifuged at 1,000 × *g* at 4 °C for 20 min. Plasma was harvested into 2-mL microcentrifuge tubes and stored at -20 °C until analysis.

Treatments were ended after the morning feeding on d 42. Weaning was then initiated as follows. Starting at the evening feeding on d 42 through d 48, calves received 1.89 L milk replacer twice daily. From d 49 through 55, calves received 1.89 L only once daily at the morning feeding. On d 56 and following, calves received no milk replacer. Starter feed, water, and cleaning remained as previously described. Health evaluations were conducted once daily at the morning feeding. Calves were vaccinated on d 53 with Bovilis Vista 5 SQ (Merck Animal Health, Omaha, NE) for IBR, BVD, BRSV, and PI3 and Bovilis Vision 7 with SPUR (Merck

Animal Health, Omaha, NE) as a clostridial vaccine including *Clostridium perfringens* Types C & D. On d 59, individual calf weight was measured.

Sample Collection and Analysis

Both milk replacer and starter samples were collected weekly and stored at -20 °C until analysis. Approximately 10 g of milk replacer from each sample was composited for analysis. Approximately 50 g of starter feed was composited and dried in a forced-air oven at 55 °C for 24 h and then ground though a 1-mm screen using a Thomas Wiley Mill (Thomas Scientific, Swedesboro, NJ). Analyses of DM (by drying at 105 °C), organic matter (OM; Undersander, 1993), crude protein (CP; N × 6.25; AOAC, 1997), ether extract, calcium (Bowers and Rains, 1988), and phosphorus (AOAC, 1997) content in milk replacer and starter feed were conducted by a commercial laboratory (SDK Laboratories; Hutchison, KS). In addition, neutral detergent fiber (Van Soest et al., 1991), acid detergent fiber (Van Soest et al., 1991), and starch (Richards et al., 1995) content were analyzed in the starter feed. Liquid milk replacer was collected twice weekly and dried for 12 h in a forced-air oven at 105 °C to determine DM content. One week of samples were inadvertently not collected; the resulting DM of the missing week was calculated as an average of the samples from the previous and following weeks.

Statistical Analysis

All data from the 3 calves that died during the trial period and 1 that was later identified as an intact male were eliminated from the data set. This resulted in 41 calves in the final data set with 13 calves for control and 14 calves each for the 1 and 2 g GAA/d treatments. Performance data was analyzed as a randomized block design using the mixed procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC) with the model including treatment as a fixed effect and block as a random effect. Orthogonal contrasts were utilized to identify linear and quadratic effects of

treatment. Health data were analyzed by first calculating the percentage of health checks where each calf resided within each of the individual categories. These percentages were then analyzed as described for the performance data. The number of electrolyte doses (1 dose = 1.89 L) within a given period of time were also analyzed as described for the performance data. Statistical significance was determined at $P \leq 0.05$ with tendencies at $0.05 < P \leq 0.15$.

Results

Growth Performance

Calves supplemented with GAA tended to have greater (linear, $P = 0.14$) BW compared to control after 42 d (Table 4.2, Figure 4.1). After weaning (d 59), GAA supplemented calves tended (linear, $P = 0.09$) to be ~7.25 kg heavier than the control group. There was a linear increase ($P = 0.005$) in hip height on d 14 with increasing GAA, but hip height did not differ among treatment groups after this point ($P > 0.15$; Table 4.2, Figure 4.2). Average daily gain (ADG), dry matter intake (DMI), and gain:feed (G:F) data is presented in Table 4.2. There was a tendency for ADG to be greater with GAA during treatment provision (linear, $P = 0.15$) and weaning (linear, $P = 0.14$) compared to control. Over the entire trial (d 0-59), ADG tended (linear, $P = 0.09$) to be 13% greater in calves supplemented GAA than in control calves.

Milk DMI did not differ among groups ($P > 0.05$). Starter DMI tended to be greater for calves supplemented with GAA (d 0 to 42, linear, $P = 0.06$; d 42 to 59, linear, $P = 0.07$; d 0 to 59, linear, $P = 0.06$). This resulted in tendencies for total DMI to be greater with GAA at all time points (d 0 to 42, linear, $P = 0.10$; d 42 to 59, linear, $P = 0.07$; d 0 to 59, linear, $P = 0.07$). However, the increase in feed consumption in response to GAA supplementation was proportional to increases in ADG, so there were no differences in G:F. Both GAA supplementation treatment groups yielded similar results during the trial.

Health Outcomes

No differences ($P > 0.05$) in health scores were observed in respiratory or fecal scores between treatment groups during treatment provision or weaning (Table 4.3). Calves receiving 1 g GAA/d tended to have a greater percentage of days at a respiratory score of 4 or more (quadratic, $P = 0.12$), but these represented only 0.5% of total days. In addition, 1 g GAA/d tended (quadratic, $P = 0.10$) to have more days at a fecal score 1 compared to other groups during the weaning period. Very little lameness occurred in our experiment and there were no significant differences for calf health scores for lameness (data not presented).

For gut health management, electrolyte was fed as described above. No differences ($P > 0.05$) in electrolyte doses were found among groups during treatment provision and no electrolyte was provided during weaning (Table 4.3).

Discussion

Growth Performance

Because BW and ADG tended to increase with GAA supplementation, this indicates GAA was absorbed and available for utilization in the body. When provided through the rumen, GAA is approximately 50% bioavailable compared to abomasal provision (Speer et al., 2020). However, because GAA was provided in the milk replacer by bottle, it would be expected to bypass the rumen via the reticular groove and reach the small intestine for absorption without concern for microbial degradation. As 1 and 2 g GAA/d yielded similar results throughout the trial, the optimal amount of supplemental GAA appears to be between 0 and 1 g GAA/d. However, more research is needed to determine this more precisely in young, milk-fed calves.

Absorbed GAA is methylated to form creatine in the liver where it can then be transported to muscle tissue for use (Wyss and Kaddurah-Daouk, 2000). Increases in plasma

GAA and creatine concentrations were observed when Holstein heifers and steers were abomasally infused with increasing levels of GAA (Ardalan et al., 2020; Ardalan et al., 2021). In humans, dietary GAA increased muscle creatine concentrations (Ostojic et al., 2016). When concurrent with exercise, greater muscle mass was also reported with creatine supplementation in humans (Brosnan and Brosnan, 2007). This indicates creatine availability is increased with GAA supplementation and this could support muscle growth. Increased concentrations of plasma insulin-like growth factor-1, and phosphorylated Akt, and phosphorylated mammalian target of rapamycin (mTOR) of the longissimus dorsi, 3 main components of the Akt/mTOR pathway, have been measured with GAA supplementation to lambs (Li et al., 2022). When activated, the Akt/mTOR pathway promotes skeletal muscle growth (Bodine et al., 2001). As well, Li et al. (2022) observed, in the longissimus dorsi of 25-kg lambs, decreased expression and concentration of myostatin, an inhibitor of myofibrillar growth, in response to GAA supplementation. In broilers, dietary GAA supplementation resulted in greater final BW after 39 d compared to a negative control; this response was similar to a natural creatine source (Michiels et al., 2012). Compared to control, BW, ADG, and G:F were greater in pigs in a 150-d study during the growing and finishing phase with GAA supplementation (Jayaraman et al., 2018); this indicates stimulation of muscle growth with GAA supplementation.

In ruminants, promising results have been observed with supplemental GAA. Improvement in BW has been observed in multiple studies with finishing bulls. After 60 d of GAA supplementation, BW was increased (Li et al., 2020; Liu et al., 2021a; Liu et al., 2021b) with a greater gain observed at 90 d when compared to control groups (Li et al., 2020). In addition, ADG has improved similarly in bulls provided various amounts of GAA from 42 to 104 d (Li et al., 2020; Li et al., 2021; Liu et al., 2021a; Liu et al., 2021b). In lambs, GAA increased

final BW after supplementation for 70 d compared to control (Li et al., 2022). This resulted in greater carcass weights with greater lean meat weight in supplemented lambs. Also, GAA provided with ruminally protected Met increased final BW, total weight gain, and ADG for the first 60 d of a 90-d study in Tan lambs (Zhang et al., 2022a). However, in comparison, another study in similar weight lambs supplemented for 100 d observed no difference between treatments in final BW or ADG (Zhang et al., 2022b). Supplementation of GAA appears to increase growth in ruminants, but there is no direct comparison for our young calves in the available literature.

As expected, milk DMI did not differ among treatments due to the limited amount provided to the calves. Calves were allowed *ad libitum* access to starter feed, and intake of starter feed tended to increase in this study when GAA was supplemented. Li et al. (2020) observed an increase in DMI of growing bulls over 104 d, with 2 levels (0.6 and 0.9 g/kg DM) of GAA yielding similar increases in intake compared to either control or a lower level of supplementation (0.3 g GAA/kg DM). In addition, average daily feed intake tended to increase in Jinjiang bulls supplemented up to 2 g GAA/kg DM for 42 d (Li et al., 2021). Ultimately, the increase in starter feed DMI we observed could be due to the increase in gain because calves need more energy to support growth. Alternatively, it could be the cause of the increase in gain because GAA led to an increase in appetite that resulted in more energy consumption driving gain.

Health Outcomes

Our calves had normal health incidences for conventional rearing of neonatal dairy calves (Hulburt and Moisa, 2016). Antioxidant status has been shown to be enhanced by GAA supplementation in pigs (Wang et al., 2012) and bulls (Li et al., 2021) which might aid in immune function. Negatively, the increased supply of GAA could consume Met for methylation

reactions (Speer et al., 2022); because Met supplementation has been shown to improve bovine immune function (Vailati-Riboni et al., 2017), we postulate that GAA could be detrimental to the actions of the immune system. However, health was not different among treatment groups, suggesting that immune function was neither greatly improved nor inhibited by GAA supplementation.

Conclusion

Supplementation of GAA to milk-fed dairy/beef cross calves appears to be beneficial. Inclusion of GAA in milk replacer fed to calves tended to increase ADG, and this was associated with increased starter feed intake. Responses were similar when GAA was supplemented at 1 or 2 g GAA/d. The benefits in gain and intake were maintained through the weaning period after treatments were no longer provided. Additional research will be necessary to better quantify the role GAA can play in increasing performance of calves fed milk replacer.

Literature Cited

AOAC. 1997. Official Methods of Analysis. 14th ed. AOAC Int., Arlington, VA.

Ardalan, M., E. D. Batista, and E. C. Titgemeyer. 2020. Effect of post-ruminal guanidinoacetic acid supplementation on creatine synthesis and plasma homocysteine concentrations in cattle. J. Anim. Sci. 98:1–9. doi:10.1093/jas/skaa072.

Ardalan, M., M. D. Miesner, C. D. Reinhardt, D. U. Thomson, C. K. Armendariz, J. S. Smith, and E. C. Titgemeyer. 2021. Effects of guanidinoacetic acid supplementation on nitrogen retention and methionine flux in cattle. J. Anim. Sci. 99:1–12. doi:10.1093/jas/skab172.

Bodine, S. C., T. N. Stitt, M. Gonzalez, W. O. Kline, G. L. Stover, R. Bauerlein, E. Zlotchenko, A. Scrimgeour, J. C. Lawrence, D. J. Glass, and G. D. Yancopoulos. 2001. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nat Cell Biol. 3:1014–1019. doi:10.1038/ncb1101-1014.

Bowers, G. N., and T. C. Rains. 1988. Measurement of total calcium in biological fluids: Flame atomic absorption spectrometry. Methods Enzymol. 158:302–319. doi:10.1016/0076-6879(88)58062-x.

Brosnan, J. T., and M. E. Brosnan. 2007. Creatine: Endogenous metabolite, dietary, and therapeutic supplement. Annu. Rev. Nutr. 27:241–261. doi:10.1146/annurev.nutr.27.061406.093621.

Brosnan, J. T., E. P. Wijekoon, L. Warford-Woolgar, N. L. Trottier, M. E. Brosnan, J. A. Brunton, and R. F. Bertolo. 2009. Creatine synthesis is a major metabolic process in neonatal piglets and has important implications for amino acid metabolism and methyl balance. J. Nutr. 139:1292–1297. doi:10.3945/jn.109.105411.

Deelen, S. M., T. L. Ollivett, D. M. Haines, and K. E. Leslie. 2014. Evaluation of a Brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves. J. Dairy Sci. 97:3838–3844. doi:10.3168/jds.2014-7939.

EFSA, (European Food Safety Authority). 2009. Safety and efficacy of guanidinoacetic acid as feed additive for chickens for fattening. The EFSA Journal. 988:1–30. doi:10.2903/j.efsa.2009.988.

FDA, (U. S. Food and Drug Administration). 2021. Code of Federal Regulations, Title 21 - Food and Drugs, Section 573.496 - Guanidinoacetic acid. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=573&showFR=1

He, D. T., X. R. Gai, L. B. Yang, J. T. Li, W. Q. Lai, X. L. Sun, and L. Y. Zhang. 2018. Effects of guanidinoacetic acid on growth performance, creatine and energy metabolism, and carcass characteristics in growing-finishing pigs. Journal of Animal Science. 96:3264–3273. doi:10.1093/jas/sky186.

Hulbert, L. E., and S. J. Moisa. 2016. Stress, immunity, and the management of calves. J. Dairy Sci. 99:3199–3216. doi:10.3168/jds.2015-10198.

Jayaraman, B., K. V. La, H. La, V. Doan, E. M. Carpena, M. Rademacher, and G. Channarayapatna. 2018. Supplementation of guanidinoacetic acid to pig diets: effects on performance, carcass characteristics, and meat quality. J. Anim. Sci. 96:2332–2341. doi:10.1093/jas/sky137.

Li, S. Y., C. Wang, Z. Z. Wu, Q. Liu, G. Guo, W. J. Huo, J. Zhang, L. Chen, L. Y. Zhang, C. X. Pei, and S. L. Zhang. 2020. Effects of guanidinoacetic acid supplementation on growth performance, nutrient digestion, rumen fermentation and blood metabolites in Angus bulls. Animal. 14:2535–2542. doi:10.1017/S1751731120001603.

Li, X., X. Liu, P. Song, J. Zhao, J. Zhang, and J. Zhao. 2022. Skeletal muscle mass, meat quality and antioxidant status in growing lambs supplemented with guanidinoacetic acid. Meat Sci. 192:1–9. doi:10.1016/j.meatsci.2022.108906.

Li, Z., H. Liang, J. Xin, L. Xu, M. Li, H. Yu, W. Zhang, Y. Ge, Y. Li, and M. Qu. 2021. Effects of dietary guanidinoacetic acid on the feed efficiency, blood measures, and meat quality of Jinjiang bulls. Front. Vet. Sci. 8:1–10. doi:10.3389/fvets.2021.684295.

Liu, C., C. Wang, J. Zhang, Q. Liu, G. Guo, W. J. Huo, C. X. Pei, L. Chen, and Y. L. Zhang. 2021. Guanididnoacetic acid and betaine supplementation have positive effects on growth performance, nutrient digestion and rumen fermentation in Angus bulls. Anim. Feed Sci. Technol. 276:1–8. doi:10.1016/j.anifeedsci.2021.114923.

Liu, Y. J., J. Z. Chen, D. H. Wang, M. J. Wu, C. Zheng, Z. Z. Wu, C. Wang, Q. Liu, J. Zhang, G. Guo, and W. J. Huo. 2021. Effects of guanidinoacetic acid and coated folic acid supplementation on growth performance, nutrient digestion and hepatic gene expression in Angus bulls. Br. J. Nutr. 126:510–517. doi:10.1017/S0007114520004341.

McGuirk, S. M., and S. F. Peek. 2014. Timely diagnosis of dairy calf respiratory disease using a standardized scoring system. Animal Health Research Reviews. 15:145–147. doi:10.1017/S1466252314000267.

Michiels, J., L. Maertens, J. Buyse, A. Lemme, M. Rademacher, N. A. Dierick, and S. De Smet. 2012. Supplementation of guanidinoacetic acid to broiler diets: Effects on performance, carcass characteristics, meat quality, and energy metabolism. Poult. Sci. 91:402–412. doi:10.3382/ps.2011-01585.

Ostojic, S. M., J. Ostojic, P. Drid, and M. Vranes. 2016. Guanidinoacetic acid versus creatine for improved brain and muscle creatine levels: A superiority pilot trial in healthy men. Appl. Physiol. Nutr. Metabl. 41:1005–1007. doi:10.1139/apnm-2016-0178.

Richards, C. J., J. F. Pederson, R. A. Britton, R. A. Stock, and C. R. Krehbiel. 1995. In vitro starch disappearance procedure modifications. Anim. Feed Sci. Technol. 55:35–45. doi:10.1016/0377-8401(95)00790-T.

Speer, H. F., K. A. Pearl, and E. C. Titgemeyer. 2020. Relative bioavailability of guanidinoacetic acid delivered ruminally or abomasally to cattle. J. Anim. Sci. 98:1–6. doi:10.1093/jas/skaa282.

Speer, H.F., M.S. Grant, M.D. Miesner, and E.C. Titgemeyer. 2022. Effect of guanidinoacetic acid supplementation on nitrogen retention and methionine methyl group flux in growing steers fed corn-based diets. J. Anim. Sci. skac283, https://doi.org/10.1093/jas/skac283

Undersander, D. J. 1993. Forage Analyses Procedures. Natl. Forage Test Assoc., Omaha, NE.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Vailati-Riboni, M., Z. Zhou, C. B. Jacometo, A. Minuti, E. Trevisi, D. N. Luchini, and J. J. Loor. 2017. Supplementation with rumen-protected methionine or choline during the transition period influences whole-blood immune response in periparturient dairy cows. J. Dairy Sci. 100:3958–3968. doi:10.3168/jds.2016-11812.

Wang, L. S., B. M. Shi, A. S. Shan, and Y. Y. Zhang. 2012. Effects of guanidinoacetic acid on growth performance, meat quality and antioxidation in growing-finishing pigs. J. Anim. Vet. Adv. 11:631–636. doi:10.3923/javaa.2012.631.636.

Wyss, M., and R. Kaddurah-Daouk. 2000. Creatine and creatine metabolism. Physiol. Rev. 80:1107–1213. doi:10.1152/physrev.2000.80.3.1107.

Zhang, J. H., H. H. Li, G. J. Zhang, Y. H. Zhang, B. Liu, S. Huang, J. Guyader, and R. Z. Zhong. 2022a. Supplementation of guanidinoacetic acid and rumen-protected methionine increased growth performance and meat quality of Tan lambs. Anim. Biosci. 1–20. doi:10.5713/ab.22.0008.

Zhang, S., C. Zang, J. Pan, C. Ma, C. Wang, X. Li, W. Cai, and K. Yang. 2022b. Effects of dietary guanidinoacetic acid on growth performance, guanidinoacetic acid absorption and creatine metabolism of lambs. PLOS ONE. 17:1–13. doi:10.1371/journal.pone.0264864.

Table 4.1. Nutrient composition of milk replacer and starter feed fed to Angus x Holstein calves

Item, % of dry matter	Milk replacer[1]	Starter feed[2]
Dry matter, % of as fed	97.00	95.16
Organic matter	94.98	93.61
Crude protein	25.33	20.55
Neutral detergent fiber	-	16.94
Acid detergent fiber	-	10.65
Starch	-	33.4
Ether extract	23.81	3.61
Calcium	0.86	1.25
Phosphorus	0.65	0.55

[1] Ingredients: dried whey, dried whey protein concentrate, dried whey product, animal fat and coconut oil (preserved with BHA and BHT), dried skimmed milk, dextrose, lecithin, dicalcium phosphate, calcium carbonate, L-lysine monohydrochloride, DL-methionine, hydrolyzed yeast, maltodextrins, iron proteinate, natural and artificial flavors, manganese proteinate, zinc proteinate, selenium yeast, copper proteinate, silicon dioxide, dried *Bifidobacterium longum* fermentation product, dried *Lactobacillus acidophilus* fermentation product, cobalt proteinate, ethylenediamine dihydriodide, vitamin A supplement, vitamin D_3 supplement, vitamin E supplement, ascorbic acid, magnesium oxide, niacin supplement, calcium pantothenate, vitamin B_{12} supplement, thiamine mononitrate, riboflavin supplement, pyridoxine hydrochloride, biotin, folic acid, choline chloride, sodium silico aluminate, mono and diglycerides of edible fats or oils.

[2] Ingredients: grain products, plant protein products, molasses products, roughage products, calcium carbonate, processed grain by-products, forage products, vegetable oil, calcium

phosphate, salt, magnesium oxide, magnesium sulfate, potassium sulfate, propionic acid, ammonium hydroxide, acetic acid, sodium carboxymethylcellulose, sodium hydroxide, vitamin E supplement, selenium yeast, benzoic acid, zinc proteinate, manganese proteinate, sorbic acid, *Aspergillus oryzae* fermentation extract, vitamin A supplement, ferrous sulfate, vitamin D_3 supplement, methylparaben, propylparaben, vitamin B_{12} supplement, riboflavin supplement, niacin supplement, thiamine mononitrate, d-calcium pantothenate, natural and artificial flavor, folic acid, biotin, copper proteinate, pyridoxine hydrochloride, ethylenediamine dihydriodide, menadione sodium bisulfite complex (source of vitamin K activity), tartaric acid, cobalt proteinate, verxite granules, choline chloride, silicon dioxide, mineral oil, natural flavor.

Table 4.2. Growth performance of milk-fed Angus x Holstein calves supplemented guanidinoacetic acid (GAA)

	GAA, g/d[1]				*P*-value	
Item	0	1	2	SEM[2]	Linear	Quadratic
n	13	14	14			
Bodyweight, kg						
Day 0	39.9	41.5	40.7	0.98	0.27	0.06
Day 42	69.3	73.8	73.8	2.46	0.14	0.40
Day 59	91.2	98.3	98.6	3.39	0.09	0.35
Hip height, cm						
Day 0	61.2	61.8	62.3	0.84	0.17	0.97
Day 42	68.7	70.1	70.2	1.00	0.20	0.51
Day 0-42 gain	7.4	8.3	7.9	0.77	0.63	0.51
Average daily gain, kg/d						
Days 0-42	0.69	0.77	0.79	0.049	0.15	0.58
Days 42-59	1.30	1.45	1.46	0.074	0.14	0.43
Days 0-59	0.86	0.97	0.98	0.049	0.09	0.45
Milk dry matter intake, kg/d						
Days 0-42	0.809	0.812	0.815	0.012	0.72	0.97
Days 42-59	0.374	0.374	0.374	0.0003	0.21	0.46
Days 0-59	0.683	0.686	0.688	0.0089	0.71	0.99
Starter dry matter intake, kg/d						
Days 0-42	0.201	0.278	0.286	0.034	0.06	0.36
Days 42-59	1.694	1.995	2.024	0.132	0.07	0.38

Days 0-59	0.631	0.772	0.786	0.060	0.06	0.36
Total dry matter intake, kg/d						
Days 0-42	1.011	1.090	1.101	0.041	0.10	0.47
Days 42-59	2.067	2.369	2.398	0.132	0.07	0.38
Days 0-59	1.315	1.458	1.475	0.064	0.07	0.39
Gain:feed, kg:kg						
Days 0-42	0.677	0.697	0.709	0.026	0.38	0.89
Days 42-59	0.626	0.621	0.609	0.016	0.47	0.89
Days 0-59	0.653	0.661	0.663	0.012	0.55	0.83

[1] Treatments provided in milk replacer for 42 days with weaning initiated on day 42.

[2] Average standard error of mean among treatments.

Table 4.3. Percentage of days of calf health scores and electrolyte dosages provided to milk-fed Angus x Holstein calves supplemented guanidinoacetic acid (GAA)

	GAA, g/d[1]				*P*-value	
Item	0	1	2	SEM[2]	Linear	Quadratic
Respiratory score[3]	------ % of days------					
Days 0-42						
0	80.9	83.2	82.5	3.0	0.71	0.67
1	13.8	10.5	12.6	1.6	0.60	0.16
2	4.2	4.4	3.4	1.3	0.68	0.71
3	1.0	1.4	1.3	0.5	0.69	0.72
4+	0.1	0.5	0.2	0.2	0.77	0.12
Days 42-59						
0	93.8	92.1	94.6	1.9	0.73	0.30
1	5.5	7.1	4.9	1.7	0.77	0.33
2	0.5	0.8	0.4	0.5	0.88	0.49
3	-	-	-			
4+	-	-	-			
Fecal score[4]						
Days 0-42						
0	36.6	40.4	38.7	2.7	0.58	0.40
1	40.2	38.6	40.4	1.6	0.92	0.37
2	18.0	15.3	17.0	1.9	0.70	0.33
3	5.2	5.6	3.9	1.2	0.43	0.47
Days 42-59						

0	63.3	56.6	58.7	3.4	0.36	0.30
1	33.5	40.4	36.4	2.6	0.44	0.10
2	3.2	2.9	4.4	2.0	0.66	0.72
3	0.0	0.0	0.4	0.2	0.25	0.49
Electrolyte, doses[5]						
Days 0-42	1.9	2.4	1.7	0.7	0.81	0.50
Days 42-59	-	-	-			

[1] Treatments provided in milk replacer for 42 days and weaning initiated on day 42.

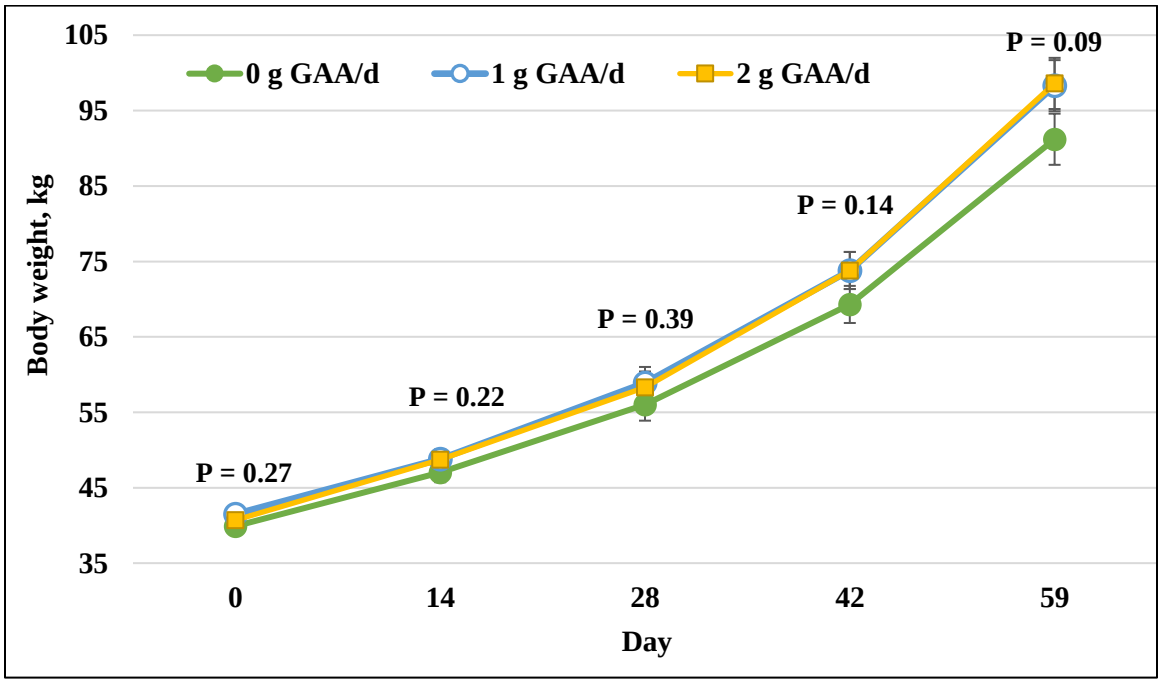
[2] Average standard error of mean among treatments.

[3] Evaluation of respiratory health was the sum of general appearance, eye and nasal discharge, and cough scores. See text for details.

[4] 0 = normal, fully formed, 1 = semi-formed, pasty, 2 = loose, but stays on top of bedding, 3 = watery, sifts through bedding.

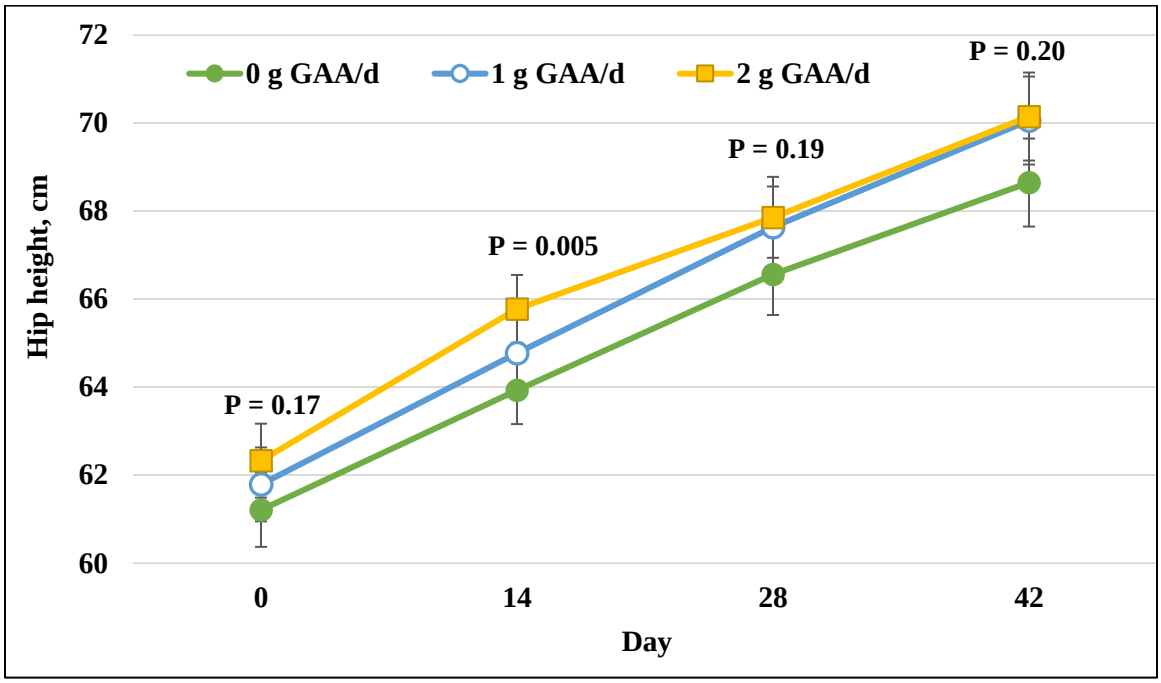
[5] Doses of electrolyte over the period, 1 dose = 1.89 L.

Figure 4.1. Effect of guanidinoacetic acid (GAA) supplementation on body weight of milk-fed calves. P-values represent linear contrast (0 g GAA/d vs. 2 g GAA/d).[1]



[1] Treatments provided in milk replacer for 42 days and weaning initiated on day 42.

Figure 4.2. Effect of guanidinoacetic acid (GAA) supplementation on hip height of milk fed calves. P-values represent linear contrast (0 g GAA/d vs. 2 g GAA/d).[1]



[1] Treatments provided in milk replacer for 42 days and weaning initiated on day 42.

Appendix A - Supplemental Tables

Table A.1. Weekly growth performance data of limit-fed steers supplemented ruminally protected lysine or blood meal during the growing phase

	Treatment[1]					Contrast *P*-value[2]		
Item	Control	Lys-3	Lys-6	BM	SEM[3]	Lys-Linear	Lys-Quad	Lys-3 vs. BM
No. of pens	8	8	8	8				
No. of steers	85	82	86	85				
Body weight, kg								
d 0	249.1	247.9	248.6	248.7	1.45	0.83	0.60	0.68
d 7	273.0	271.3	272.2	270.4	2.33	0.81	0.64	0.78
d 14	286.9	288.6	286.7	284.6	2.00	0.95	0.45	0.16
d 21	297.1	297.0	295.7	291.6	2.34	0.68	0.84	0.11
d 28	306.9	309.7	307.2	304.3	2.40	0.93	0.38	0.12
d 35	321.5	322.8	320.8	319.0	2.39	0.84	0.59	0.26
d 42	329.4	331.4	330.2	327.4	2.89	0.85	0.66	0.34
d 49	346.2	350.1	345.5	341.1	2.97	0.88	0.26	0.04
d 56	358.9	363.7	358.4	354.0	2.90	0.90	0.17	0.03
d 63	369.7	374.6	370.7	365.5	3.20	0.83	0.27	0.06
d 70	385.2	388.4	384.7	379.9	3.31	0.92	0.41	0.08
d 77	393.7	401.3	397.9	392.8	3.87	0.45	0.26	0.13

Average daily gain, kg/d								
d 0 to 7	3.42	3.34	3.37	3.09	0.255	0.89	0.86	0.49
d 0 to 14	2.70	2.91	2.72	2.56	0.083	0.88	0.06	0.01
d 0 to 21	2.29	2.34	2.24	2.04	0.074	0.66	0.42	0.01
d 0 to 28	2.07	2.21	2.09	1.98	0.059	0.76	0.09	0.01
d 0 to 35	2.07	2.14	2.06	2.01	0.054	0.94	0.28	0.09
d 0 to 42	1.91	1.99	1.94	1.87	0.051	0.70	0.35	0.12
d 0 to 49	1.98	2.09	1.98	1.89	0.048	0.95	0.08	0.01
d 0 to 56	1.96	2.07	1.96	1.88	0.041	0.98	0.04	<0.01
d 0 to 63	1.92	2.01	1.94	1.85	0.041	0.70	0.10	0.01
d 0 to 70	1.95	2.01	1.94	1.87	0.038	0.99	0.20	0.02
d 0 to 77	1.88	1.99	1.94	1.87	0.042	0.32	0.12	0.05
Dry matter intake, kg/d								
d 0 to 7	5.64	5.65	5.74	5.80	0.090	0.41	0.69	0.25
d 0 to 14	6.07	6.05	6.10	6.11	0.064	0.73	0.64	0.50
d 0 to 21	6.32	6.31	6.33	6.33	0.054	0.90	0.84	0.84
d 0 to 28	6.54	6.54	6.54	6.51	0.054	1.00	0.97	0.68
d 0 to 35	6.71	6.72	6.71	6.68	0.052	0.97	0.89	0.57
d 0 to 42	6.87	6.90	6.88	6.85	0.054	0.90	0.74	0.56
d 0 to 49	7.03	7.07	7.05	7.01	0.055	0.87	0.66	0.46
d 0 to 56	7.19	7.23	7.20	7.15	0.057	0.89	0.61	0.33
d 0 to 63	7.35	7.40	7.36	7.31	0.058	0.88	0.51	0.27
d 0 to 70	7.49	7.56	7.51	7.47	0.060	0.80	0.44	0.28

d 0 to 77	7.66	7.73	7.68	7.63	0.061	0.77	0.41	0.23
Gain:feed, kg:kg								
d 0 to 7	0.644	0.623	0.599	0.543	0.0526	0.55	0.98	0.29
d 0 to 14	0.453	0.489	0.451	0.424	0.0137	0.90	0.04	<0.01
d 0 to 21	0.364	0.374	0.357	0.325	0.0105	0.62	0.29	<0.01
d 0 to 28	0.318	0.341	0.322	0.307	0.0084	0.73	0.05	0.01
d 0 to 35	0.309	0.321	0.309	0.303	0.0074	0.96	0.18	0.08
d 0 to 42	0.280	0.290	0.284	0.274	0.0061	0.65	0.29	0.08
d 0 to 49	0.283	0.297	0.283	0.270	0.0052	0.90	0.04	<0.01
d 0 to 56	0.275	0.288	0.274	0.265	0.0043	0.94	0.02	<0.01
d 0 to 63	0.262	0.273	0.264	0.255	0.0041	0.65	0.05	<0.01
d 0 to 70	0.261	0.267	0.260	0.252	0.0038	0.88	0.18	0.01
d 0 to 77	0.247	0.259	0.254	0.247	0.0040	0.25	0.08	0.04

[1] Control = no supplemental amino acids/protein, Lys-3 = 3 g/d metabolizable Lys from Smartamine ML, Lys-6 = 6 g/d metabolizable Lys from Smartamine ML, BM = supplemental blood meal at 0.89% of dietary dry matter plus 2 g/d of metabolizable Met provided from Smartamine M.

[2] Lys-Linear = control vs. Lys-6, Lys-Quad = Lys-3 vs. average of control and Lys-6.

[3] Average standard error of mean of treatments.

Table A.2. Day and treatment x day interaction of plasma amino acid concentrations of limit-fed steers supplemented ruminally protected lysine or blood meal during the growing phase

	Treatment[1] – Day 14				Treatment – Day 77					*P*-value	
Amino acid, μM	Control	Lys-3	Lys-6	BM	Control	Lys-3	Lys-6	BM	SEM[2]	Day	Trt x Day
No. of pens	8	8	8	8	8	8	8	8			
Alanine	229.8	236.5	230.9	221.2	236.4	241.5	233.3	231.4	6.60	0.11	0.90
Arginine	146.3	147.7	152.5	146.5	150.4	152.5	155.1	151.0	4.32	0.15	0.99
Asparagine	60.0	56.6	57.2	59.9	67.7	65.5	68.0	66.9	1.54	<0.001	0.42
Aspartate	15.1	15.9	15.6	15.3	13.6	12.7	12.2	12.4	0.45	<0.0001	0.13
Glutamate	255.5	256.2	261.5	253.4	273.4	269.5	269.8	265.1	4.17	<0.0001	0.51
Glutamine	111.8	111.0	117.8	102.7	96.7	97.9	99.8	96.0	3.84	<0.0001	0.38
Glycine	365.7	361.2	363.9	317.5	315.1	316.6	325.9	290.1	9.93	<0.0001	0.65
Histidine	29.5	29.1	28.8	27.8	27.8	26.4	28.0	26.1	0.99	0.01	0.79
Isoleucine	97.1	99.7	99.5	96.4	117.2	116.1	116.1	113.8	2.93	<0.0001	0.87
Leucine	158.3	162.7	162.9	169.2	202.9	201.1	203.7	210.8	5.02	<0.0001	0.90

Lysine	88.8	89.0	91.5	97.8	121.4	113.3	117.8	117.1	3.89	<0.0001	0.38
Methionine	36.8	38.3	39.3	39.6	32.3	30.9	32.7	32.5	1.54	<0.0001	0.81
Ornithine	70.9	68.2	71.1	74.4	86.7	84.8	88.0	86.6	2.43	<0.0001	0.63
Phenylalanine	56.2	56.7	55.8	55.7	60.2	61.1	60.3	62.4	1.76	0.0001	0.82
Proline	81.0	81.9	82.0	78.8	80.8	79.5	83.7	80.4	2.60	0.91	0.74
Serine	36.6	40.8	38.9	40.9	52.1	54.2	53.8	56.8	2.13	<0.0001	0.95
Taurine	39.6	41.9	40.4	38.2	35.1	34.3	35.3	34.3	1.77	0.0005	0.77
Threonine	75.8	79.1	76.1	77.0	70.6	69.3	69.8	69.3	2.60	0.0001	0.77
Tryptophan	36.1	38.2	38.2	36.9	50.9	51.9	52.1	50.1	1.57	<0.0001	0.95
Tyrosine	70.5	69.3	65.8	65.7	68.1	65.8	64.8	63.6	2.73	0.30	0.98
Valine	227.7	240.5	238.0	252.3	281.2	288.9	289.1	304.6	7.24	<0.0001	0.97
Total	2286.1	2320.3	2327.6	2266.8	2440.5	2433.7	2459.0	2422.0	40.80	<0.0001	0.92

[1] Control = no supplemental amino acids/protein, Lys-3 = 3 g/d metabolizable Lys from Smartamine ML, Lys-6 = 6 g/d metabolizable Lys from Smartamine ML, BM = supplemental blood meal at 0.89% of dietary dry matter plus 2 g/d of metabolizable Met provided from Smartamine M.

[2] Average standard error of mean among treatments.

Table A.3. Average economic value of steers supplemented ruminally protected lysine or blood meal during the growing phase slaughtered on March 30, 2021 and April 20, 2021, based on U.S. Premium Beef Base Grid

	Treatment[1]					Contrast *P*-value[2]		
Item	Control	Lys-3	Lys-6	BM	SEM[3]	Lys-Linear	Lys-Quad	Lys-3 vs. BM
No. of steers	81	79	82	81				
Value, $/steer	1835	1834	1843	1828	17.6	0.72	0.80	0.81
Value, $/45.5 kg carcass weight	191.91	190.20	189.85	191.34	0.77	0.03	0.38	0.21

[1] Treatments provided during the 77-day growing phase, but not during the finishing phase. Control = no supplemental amino acids/protein, Lys-3 = 3 g/d metabolizable Lys from Smartamine ML, Lys-6 = 6 g/d metabolizable Lys from Smartamine ML, BM = supplemental blood meal at 0.89% of dietary dry matter plus 2 g/d of metabolizable Met provided from Smartamine M.

[2] Lys-Linear = control vs. Lys-6, Lys-Quad = Lys-3 vs. average of control and Lys-6.

[3] Average standard error of mean among treatments.

Table A.4. Bi-weekly growth performance of milk-fed Angus x Holstein calves supplemented guanidinoacetic acid (GAA)

	GAA, g/d[1]				*P*-value	
Item	0	1	2	SEM[2]	Linear	Quadratic
n	13	14	14			
Bodyweight, kg						
Day 0	39.9	41.5	40.7	0.98	0.27	0.06
Day 14	47.0	48.8	48.7	1.27	0.22	0.41
Day 28	56.0	58.9	58.3	2.11	0.39	0.44
Day 42	69.3	73.8	73.8	2.46	0.14	0.40
Day 59	91.2	98.3	98.6	3.39	0.09	0.35
Hip height, cm						
Day 0	61.2	61.8	62.3	0.84	0.17	0.97
Day 14	63.9	64.8	65.8	0.77	0.005	0.88
Day 28	66.6	67.6	67.9	0.92	0.19	0.61
Day 42	68.7	70.1	70.2	1.00	0.20	0.51
Hip height gain, cm						
Day 0-14	2.8	3.0	3.5	0.49	0.23	0.69
Day 14-28	2.5	2.8	2.0	0.53	0.51	0.36
Day 0-28	5.3	5.8	5.6	0.68	0.80	0.70
Day 28-42	2.0	2.5	2.3	0.57	0.73	0.67
Day 0-42	7.4	8.3	7.9	0.77	0.63	0.51
Average daily gain, kg/d						

Days 0-14	0.50	0.53	0.56	0.055	0.38	0.97
Days 14-28	0.63	0.72	0.68	0.097	0.68	0.56
Days 0-28	0.56	0.63	0.62	0.059	0.47	0.63
Days 28-42	0.95	1.06	1.11	0.052	0.02	0.61
Days 0-42	0.69	0.77	0.79	0.049	0.15	0.58
Days 42-59	1.30	1.45	1.46	0.074	0.14	0.43
Days 0-59	0.86	0.97	0.98	0.049	0.09	0.45
Milk dry matter intake, kg/d						
Days 0-14	0.775	0.795	0.777	0.027	0.98	0.56
Days 14-28	0.807	0.795	0.828	0.024	0.55	0.46
Days 0-28	0.791	0.795	0.802	0.019	0.68	0.95
Days 28-42	0.844	0.845	0.841	0.003	0.49	0.64
Days 0-42	0.809	0.812	0.815	0.012	0.72	0.97
Days 42-59	0.374	0.374	0.374	0.0003	0.21	0.46
Days 0-59	0.683	0.686	0.688	0.0089	0.71	0.99
Starter dry matter intake, kg/d						
Days 0-14	0.037	0.044	0.057	0.009	0.05	0.76
Days 14-28	0.130	0.193	0.185	0.030	0.19	0.33
Days 0-28	0.083	0.119	0.121	0.019	0.14	0.44
Days 28-42	0.436	0.597	0.615	0.067	0.04	0.34
Days 0-42	0.201	0.278	0.286	0.034	0.06	0.36
Days 42-59	1.694	1.995	2.024	0.132	0.07	0.38
Days 0-59	0.631	0.772	0.786	0.060	0.06	0.36

Total dry matter intake, kg/d						
Days 0-14	0.813	0.840	0.833	0.030	0.64	0.64
Days 14-28	0.937	0.988	1.013	0.047	0.26	0.83
Days 0-28	0.875	0.914	0.923	0.032	0.29	0.70
Days 28-42	1.280	1.441	1.456	0.068	0.05	0.33
Days 0-42	1.011	1.090	1.101	0.041	0.10	0.47
Days 42-59	2.067	2.369	2.398	0.132	0.07	0.38
Days 0-59	1.315	1.458	1.475	0.064	0.07	0.39
Gain:feed, kg:kg						
Days 0-14	0.623	0.603	0.671	0.062	0.58	0.56
Days 14-28	0.628	0.648	0.651	0.102	0.88	0.95
Days 0-28	0.629	0.664	0.662	0.053	0.67	0.78
Days 28-42	0.751	0.741	0.767	0.025	0.65	0.55
Days 0-42	0.677	0.697	0.709	0.026	0.38	0.89
Days 42-59	0.626	0.621	0.609	0.016	0.47	0.89
Days 0-59	0.653	0.661	0.663	0.012	0.55	0.83

[1] Treatments provided in milk replacer for 42 days and weaning initiated on day 42.

[2] Average standard error of mean among treatments.

Table A.5. Bi-weekly percentage of days of calf health scores days and electrolyte dosages provided to milk-fed Angus x Holstein calves supplemented guanidinoacetic acid (GAA)

	GAA, g/d[1]				*P*-value	
Item	0	1	2	SEM[2]	Linear	Quadratic
Respiratory score[3]	------ % of days------					
Days 0-14						
0	84.9	88.8	85.5	3.1	0.89	0.34
1	10.2	5.6	8.2	1.4	0.34	0.04
2	3.3	3.8	3.4	1.8	0.96	0.83
3	1.6	1.3	2.6	0.8	0.43	0.41
4+	< 0.01	0.51	0.26	0.3	0.60	0.36
Days 14-28						
0	80.2	80.4	81.1	5.1	0.90	0.96
1	13.2	11.2	13.5	2.5	0.93	0.49
2	5.8	5.9	4.3	2.2	0.66	0.77
3	0.8	2.0	0.8	0.8	0.96	0.23
4+	< 0.01	0.51	0.26	0.3	0.49	0.23
Days 0-28						
0	82.6	84.6	83.3	3.5	0.88	0.70
1	11.7	8.4	10.9	1.7	0.75	0.17
2	4.5	4.8	3.9	1.6	0.78	0.75
3	1.2	1.7	1.7	0.6	0.63	0.79
4+	< 0.01	0.51	0.26	0.2	0.37	0.13
Days 28-42						

0	78.0	80.7	81.0	3.1	0.44	0.72
1	17.7	14.5	16.0	2.2	0.54	0.33
2	3.6	3.6	2.6	1.2	0.55	0.73
3	0.5	0.8	0.5	0.4	0.95	0.66
4+	0.27	0.51	0.0	0.3	0.57	0.37
Days 0-42						
0	80.9	83.2	82.5	3.0	0.71	0.67
1	13.8	10.5	12.6	1.6	0.60	0.16
2	4.2	4.4	3.4	1.3	0.68	0.71
3	1.0	1.4	1.3	0.5	0.69	0.72
4+	0.1	0.5	0.2	0.2	0.77	0.12
Days 42-59						
0	93.8	92.1	94.6	1.9	0.73	0.30
1	5.5	7.1	4.9	1.7	0.77	0.33
2	0.5	0.8	0.4	0.5	0.88	0.49
3	-	-	-			
4+	-	-	-			
Days 0-59						
0	83.2	84.7	84.6	2.4	0.68	0.78
1	12.3	9.9	11.3	1.3	0.57	0.24
2	3.6	3.8	2.9	1.1	0.67	0.66
3	0.8	1.1	1.1	0.4	0.70	0.72
4+	0.1	0.4	0.1	0.2	0.77	0.12

Fecal score[4]						
Days 0-14						
0	28.2	33.2	30.0	3.0	0.65	0.23
1	43.8	41.2	46.7	1.9	0.45	0.21
2	23.3	20.0	20.5	2.9	0.44	0.53
3	4.7	5.7	2.9	1.4	0.39	0.29
Days 14-28						
0	25.0	28.8	28.8	4.2	0.53	0.71
1	40.4	39.1	39.0	3.0	0.73	0.81
2	24.5	20.7	25.0	3.3	0.91	0.33
3	10.2	11.2	7.1	2.6	0.41	0.42
Days 0-28						
0	26.6	31.0	29.3	3.0	0.53	0.41
1	42.1	40.2	42.8	2.0	0.81	0.37
2	23.9	20.3	22.8	2.4	0.73	0.29
3	7.4	8.5	5.1	1.8	0.36	0.32
Days 28-42						
0	56.5	59.2	57.4	3.2	0.85	0.57
1	36.4	35.5	35.8	2.3	0.84	0.84
2	6.1	5.3	5.3	1.9	0.74	0.85
3	0.8	0.0	1.5	0.7	0.48	0.18
Days 0-42						
0	36.6	40.4	38.7	2.7	0.58	0.40

1	40.2	38.6	40.4	1.6	0.92	0.37
2	18.0	15.3	17.0	1.9	0.70	0.33
3	5.2	5.6	3.9	1.2	0.43	0.47
Days 42-59						
0	63.3	56.6	58.7	3.4	0.36	0.30
1	33.5	40.4	36.4	2.6	0.44	0.10
2	3.2	2.9	4.4	2.0	0.66	0.72
3	0.0	0.0	0.4	0.2	0.25	0.49
Days 0-59						
0	41.0	43.1	42.1	2.5	0.77	0.60
1	39.1	38.9	39.7	1.6	0.74	0.78
2	15.5	13.3	14.9	1.6	0.77	0.28
3	4.4	4.7	3.3	1.0	0.47	0.49
Electrolyte, doses[5]						
Days 0-14	0.8	1.0	0.8	0.6	0.91	0.71
Days 14-28	0.6	1.2	0.5	0.4	0.88	0.20
Days 0-28	1.4	2.2	1.3	0.6	0.85	0.29
Days 28-42	0.5	0.2	0.4	0.2	0.83	0.38
Days 0-42	1.9	2.4	1.7	0.7	0.81	0.50
Days 42-59	-	-	-			
Days 0-59	1.9	2.4	1.7	0.7	0.81	0.50

[1] Treatments provided in milk replacer for 42 days and weaning initiated on day 42.

[2] Average standard error of mean among treatments.

[3] Evaluation of respiratory health was the sum of general appearance, eye and nasal discharge, and cough scores.

[4] 0 = normal, fully formed, 1 = semi-formed, pasty, 2 = loose, but stays on top of bedding, 3 = watery, sifts through bedding.

[5] Average daily doses of electrolyte, 1 dose = 1.89 L.