Limiting amino acids for growing cattle fed diets based on corn and corn gluten feed

by

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Abstract

Ruminants rely on microbial protein as a significant source of amino acids (AA), yet ambitious production goals may increase AA requirements beyond supplies of AA provided by microbial protein. Providing growing cattle with adequate quantities of post-ruminal, absorbable AA is essential for optimizing performance. Two experiments were conducted to determine which AA were limiting for Angus × Holstein crossbred steers fed a corn-based diet. In Experiment 1, seven ruminally cannulated steers (192 kg) were used in a 6 × 6 Latin square. Treatments were infused abomasally across 6-d periods and included: 1) control (water infusion); 2) 6 g/d lysine (6Lys); 3) 2 g/d methionine (2Met); 4) 6 g/d lysine plus 2 g/d methionine (6Lys2Met); 5) 12 g/d lysine plus 4 g/d methionine (12Lys4Met); and 6) 12 g/d lysine plus 4 g/d each of methionine, histidine, leucine, isoleucine, valine, threonine, arginine, phenylalanine, and 1 g/d tryptophan (MIX). Steers were fed 4.5 kg/d (DM basis) of a corn-based diet (57% dry-rolled corn, 23% corn gluten feed, and 16% grass hay). Periods included 2-d adaptations and 4 d for total fecal and urine collections to measure nitrogen retention. Data were analyzed as a Latin square with fixed effects of treatment and period and random effect of steer. Means were separated with pairwise t-tests. Compared to nitrogen retention of control (26.0 g/d), MIX (33.2 g/d; $P<0.01$), 6Lys2Met (29.5 g/d, $P=0.01$), and 12Lys4Met (29.3 g/d; $P=0.02$) increased nitrogen retention. Because 6Lys2Met and 12Lys4Met yielded similar improvements in nitrogen retention, supplemental requirements were not greater than 6 g/d lysine (Lys) and 2 g/d methionine (Met) when no other AA were provided. The greater response for MIX than for 12Lys4Met ($P=0.01$) demonstrates at least one other essential AA became limiting once supplemental requirements for Lys and Met were met. Positive nitrogen retention responses to 6Lys2Met but not to 6Lys ($P=0.14$) or 2Met ($P=0.59$) suggests that Lys and Met were

colimiting. Plasma Lys concentrations were greater (P<0.05) than control (73.8 μM) for 12Lys4Met (111.2 μM) and MIX (104.6 μM). Lys requirements were likely met somewhere between 6 and 12 g/d as increases in plasma Lys were much greater when post-ruminal Lys increased from 6 to 12 g/d than when post-ruminal Lys increased from 0 to 6 g/d. Plasma Met was greater (P<0.05) for 2Met (34.0 μM) than for control (29.0 μM); however, plasma Met for 6Lys2Met (31.2 μM) was not different (P=0.43) than control, suggesting increased utilization of Met for protein deposition. Utilization efficiencies of Lys and Met were greater for 6Lys2Met and MIX than for 6Lys or 2Met alone. Efficiency was less for 12Lys4Met than for 6Lys2Met, indicating supplemental Lys and Met requirements were less than amounts provided by 12 Lys4Met. As a whole, data demonstrated that Lys and Met were co-limiting for growing cattle fed a corn-based diet, and at least one additional essential AA was also being limiting. Experiment 2 was similar to Experiment 1 and used six of the same crossbred steers (238 kg) fed 5.3 kg/d (DM basis) of the same diet. The six abomasally infused treatments included: 1) MIX; 2) MIX without lysine (–Lys); 3) MIX without methionine (–Met); 4) MIX without histidine (–His); 5) MIX without leucine, isoleucine, and valine (–BCAA); and 6) MIX without threonine and tryptophan (–Thr/Trp). Removal of individual AA from MIX resulted in at least numerically decreased plasma concentration of that individual AA. Plasma concentrations for most AA except lysine and methionine were numerically greater for –Lys and –Met than for MIX. Nitrogen retention (42.2 g/d) was numerically greatest for steers receiving MIX. Nitrogen retention was lower for –Lys (35.1 g/d; P=0.11), –Met (35.6 g/d; P=0.11), and –Trp/Thr (36.6 g/d; P=0.17) than MIX. Nitrogen retention was not altered for –BCAA (39.7 g/d; P=0.51) or –His (40.5 g/d; P=0.72). Lysine and methionine appeared to be the first-limiting AA for growing

cattle fed a corn-based diet, and the extent of their limitation appears to be similar. Tryptophan and/or threonine may have been additionally limiting.

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Acknowledgments

As every graduate student comes to find, graduate school is a challenge that demands persistence, long hours, and the support of many. Nearing the end of my program at Kansas State, I have had time to reflect on the last two years and have realized how fortunate I have been to have the guidance, encouragement, and patience of many. A special thanks goes out to my major professor, Dr. Evan Titgemeyer, whose patience and willingness to share his vast knowledge have been invaluable. I will be leaving with a greater understanding of the world around me, and more importantly, stronger critical thinking skills that will serve me beyond this experience. For that, I am truly grateful. I am also grateful to my fellow graduate students for their help and friendship, my committee members for their guidance and open-door policy, and everyone at the Stocker Unit for their assistance in making my projects possible. A sincere thanks to my family for their unwavering support and to my fiancée for her unconditional love. Thank you all for your encouragement and kindness as they have made all the difference.

Dedication

To my father, whose unwavering work ethic and wisdom have guided me.

To my mother, whose love and belief in me has been a source of inspiration.

To my fiancé, whose patience and love have never ceased to support me.

Thank you all for your boundless encouragement.

Chapter I – Review of Literature

Introduction

Ruminants are unique in their reliance on fermentation to provide a source of amino acids (AA) that otherwise may not be found in the diet. AA of microbial origin usually make up a large portion of the total AA reaching the small intestine and can be in excess of animal maintenance requirements. Beef cattle production systems often incorporate ambitious performance goals; in these scenarios, microbial protein may not be sufficient to fulfill animal AA requirements (Richardson and Hatfield, 1978). For this reason, animals may be partially reliant on dietary AA sources that escape ruminal degradation. Optimizing both microbial protein synthesis and dietary escape protein is essential for peak performance and lean deposition. Extensive research has been conducted on methods to alter the quantity and quality of AA reaching the small intestine for absorption; however, practical use and execution relies heavily on our ability to predict AA requirements, a problematic task when working with ruminants (Merchen and Titgemeyer, 1992).

The NASEM (2016) model uses metabolizable protein rather than individual AA to predict requirements in beef cattle. Under these conditions, individual AA requirements may not be met or wasteful amounts of AA may be fed. Predicting individual AA requirements would offer substantial advantages over this system. However, factors such as animal weight and production level, diet, AA supplementation strategy, and predictor variables have not remained constant amongst lab groups attempting to do so, leading to contradictory results. Estimating animal requirements for any nutrient is, by nature, difficult. AA are no exception as the full extent to which an AA is limiting cannot be measured unless all other nutrients are provided in excess of requirements, a difficult task when trying to maintain realistic experimental conditions

(Zimmerman and Scott, 1965). Despite variation among studies, methionine and lysine have frequently been shown to be most limiting (Richardson and Hatfield, 1978; Oke et al., 1986; Klemesrud et al., 2000). This review outlines the factors affecting the prediction of AA requirements, provides current estimations of AA requirements in cattle, and evaluates each AA that has been identified as limiting or partially limiting in growing cattle.

Factors Affecting Prediction of Amino Acid Requirements

The AA requirements of growing cattle, as determined by nitrogen retention and plasma AA concentrations, vary widely. These discrepancies can be partly attributed to differences in animal weight and production levels; conceptually, larger animals growing at a faster rate would require greater quantities of AA. Fenderson and Bergen (1975) and Hutton and Annison (1972) revealed that AA requirements likely increase as steer weight increases. Average steer weights of 274 kg and 200 kg, with similar growth performance, led to corresponding estimates of methionine requirements of 14.9 g/d and 12.9 g/d respectively (Fenderson and Bergen, 1975). Research conducted by Williams and Smith (1974) further validates this notion, as they found the methionine requirements (9.8 g/d) of 110 to 160 kg steers to be lower than those reported by the aforementioned research. Klemesrud et al. (2000) and Wilkerson et al. (1993) examined steers of similar size (269 and 253 kg) and growth performance and estimated methionine requirements to be 11.6 g/d and 11.9 g/d, respectively. These papers also reported the methionine requirements as a percentage of metabolizable protein requirements (3.1% and 3.0%). However, incongruities arise when comparing the works of Tzeng et al. (1974) and Williams and Hewitt (1979). Both groups examined steers around 50 kg to estimate lysine requirements and reported somewhat different results (9.8 and 7.8 g/d), suggesting AA requirements are partially reliant

upon other circumstantial variables. The differences in average daily gain (ADG), reported as 0.70 and 0.25 kg/d, indicate growth rate is a likely suspect for the discrepancy. Later work conducted by Tzeng and Davis (1980) suggests that not all variation can be accounted for by weight and growth performance as smaller steers (25 to 50 kg) with similar ADG (0.70 kg/d) were found to have greater lysine requirements (12.4 to 14.3 kg/d) than previously determined. This is significant as it underscores that AA requirements are dependent upon a complex array of variables.

A share of the variation in predicting AA requirements can be attributed to differences in experimental approaches rather than differences amongst animals. A large contributor is experimental diets and level of intake. Diet composition influences the extent to which an AA is limiting as protein sources are variable in AA quality and quantity. As an example, soybean meal contains relatively small quantities of methionine and corn gluten meal is unlikely to provide sufficient quantities of lysine (Donahue et al. 1985; Huang et al. 2005). Ideally, intake and composition would facilitate diets being deficient in the AA of interest while supplying ample quantities of the remaining nutrients. This approach has been more effectively executed in research with pre-ruminant calves. To estimate the AA requirements of pre-ruminant calves, Tzeng and Davis (1980) created a semi-purified diet patterned to resemble whole milk by replacing milk protein with an AA mixture. They were able provide adequate nutrients alongside graded levels of lysine and methionine to measure a more complete response. Similarly, Williams and Hewitt (1979) designed a diet to resemble the composition of whole milk excluding lysine, and Foldager et al. (1977) formulated a diet made to resemble whole milk excluding methionine. Under these experimental conditions, milk contains an excess of the remaining essential amino acids beyond requirements (Bittar et al., 2018), ensuring the AA of

interest is isolated as a limiting nutrient. This, paired with the limited dietary fermentation encountered with a pre-ruminant calf, allows for much more precise predictions than for fully functioning ruminants.

Assessment of research with ruminating cattle highlights that significant variation in diet composition and level of intake can reduce consistency in predicting AA requirements. Wilkerson and Klopfenstein (1993) compiled data from 11 separate experiments, in which experimental animals were fed diets consisting of 75% to 93% roughage, with variable protein sources including soybean meal, corn gluten meal, urea, blood meal, feather meal, and dry distiller grains. The average dry matter intake (DMI) among these studies ranged from 4.2 to 7.5 kg/d. Later work by Klemesrud and Klopfenstein (2000) utilized diets fed at 2% of body weight (BW) daily consisting of 44% sorghum silage, 44% corn cobs and one of two protein sources: meat and bone meal or corn gluten meal. Williams and Smith (1974) investigated the effects of three high-roughage diets with varying inclusions of groundnut meal, corn gluten meal, and corn starch. As expected, they demonstrated that plasma AA and plasma urea concentrations were dependent on both total protein intake as well as the nature of the protein. This is important as AA from the diet and from microbial origin must be considered when estimating AA requirements. Fenderson and Bergen (1975) fed a concentrate rich diet (fed at 3% of BW daily) consisting of 62% corn and oat grain. Clearly, dietary input among lab groups estimating requirements has been inconsistent, and the combination of variable DMI, roughage content, and protein sources of differing AA profiles complicates the repeatability of these estimates. Therefore, accurate estimation of post-ruminal AA flow is essential for making reasonable comparisons among experiments.

Wilkerson and Klopfenstein (1993) estimated the energy and protein supplied by each feed ingredient and then used the equation developed by Burroughs et al. (1974) for predicting microbial protein production. This approach likely provided an accurate estimate of the AA reaching the abomasum as their proposed requirements for supplemental methionine aligned closely with subsequent findings from the same research group (3.0% and 3.1% of metabolizable protein). Klemesrud and Klopfenstein (2000) collected abomasal contents at slaughter and analyzed them to identify the post-ruminal AA composition. This method of direct measurement validated the approach of Wilkerson and Klopfenstein (1993) as it is a useful means of assessing AA flow. Another approach was taken by Williams and Smith (1974) when they dosed steers with chromic oxide and then estimated metabolizable protein from the AA:chromic oxide ratios in abomasal samples. Fenderson and Bergen (1975) used a similar marker technique with chromic oxide and abomasal sampling. Both Williams and Smith (1974) and Fenderson and Bergen (1975) reached similar conclusions as they predicted methionine requirements to be 0.23 to 0.26 g/kg $BW^{0.73}$ and 0.25 g/kg $BW^{0.73}$, respectively. Given the limited number of studies quantifying the AA requirements of growing cattle, additional research is needed to establish requirements for cattle under various dietary inputs.

A common approach for determining AA requirements is to feed a diet deficient in a specific AA and incrementally increase its supply until further supplementation no longer improves the variable of interest. For effective use of this method, AA need to be supplied post-ruminally to avoid manipulation by ruminal microbes. There are many ways to provide AA post-ruminally; the most common uses a peristaltic pump to infuse a solution containing the AA into the abomasum via ruminal canulation (Williams and Smith, 1974; Fenderson and Bergen, 1975; Burris et al., 1976). This approach allows for bypass of the rumen and complete availability once

in the abomasum. Klemesrud and Klopfenstein (2000) utilized another method involving a rumen-protected product introduced in the feed. Variation is introduced with this approach and may stem from the inconsistency of the product and the extent to which the product is degraded ruminally or passed to feces undigested. Underestimation of degradation of the product by ruminal microbes and overestimation of post-ruminal availability would lead to an overestimation of AA requirements. A third method was utilized by Hall et al. (1974) to determine the limiting AA of young bull calves. They supplied AA via intraperitoneal injection and measured the response of plasma AA concentrations and nitrogen retention. This method has not been validated for use in cattle, and any conclusions drawn from it should be interpreted with caution. Notably, AA supplied by intraperitoneal injection would bypass normal splanchnic metabolism and thereby make a greater proportion of AA available for utilization by the animal.

To accurately predict AA requirements researchers must rely on predictor variables such as plasma AA concentrations, nitrogen balance, or growth performance. Plasma AA concentrations are used with the understanding that increasing the supply of a limiting amino acid will have minimal effect until the supply exceeds the requirements, at which point plasma concentrations will rapidly rise (Zimmerman and Scott, 1965; Stockland et al., 1970). This is commonly referred to as the plasma breakpoint and is often used to estimate AA requirements. Conceptually, nitrogen retention will increase with increased supply of a limiting AA until another nutrient becomes limiting or the animal's genetic potential is reached, at which point a plateau is reached. Liebig's Law of the Minimum barrel (Liebig and Playfair, 1840) can be used to demonstrate this concept as a barrel's capacity cannot exceed the height of the smallest stave. Similarly, under certain conditions, growth performance (ADG, gain:feed [G:F]) is expected to increase with supply of a limiting AA until the point in which another nutrient or becomes

limiting or the maximal growth potential is met. Although predictions of AA requirements based on plasma AA profiles are useful indicators, they have been shown to produce contradictory results.

Williams and Hewitt (1979) estimated lysine requirements for pre-ruminant calves using plasma AA concentrations, nitrogen balance, and plasma urea concentrations. Estimates were 7.5, 7.2, and 8.5 g/d, respectively. Interestingly, estimations using nitrogen retention and plasma AA concentrations were markedly lower than estimates based upon plasma urea. As a limiting AA is supplied, plasma urea concentrations decrease until the AA requirement is met. This decrease is attributable to the increased use of AA for protein deposition and thus, decreased AA degradation required to remove excess AA nitrogen from the body. It is unclear why plasma urea concentrations estimated lysine requirements to be greater than estimates based on nitrogen retention and plasma AA concentrations. Plasma urea concentrations have been used to estimate AA requirements in sows and lambs (Lewis and Speer, 1973; Kirk and Walker, 1976) and the method remains conceptually sound. However, despite the clear two-phase response curve reported by Williams and Hewitt (1974), caution should be used as this method has not been extensively validated for use in cattle. They also used average daily gain (ADG) as a predictor and estimated lysine requirements to be 8.1 g/d, and they attributed the inflated value to the difficulties associated with measuring a low growth rate of 0.25 kg/d in small calves. The lab group considered each predictor, nitrogen balance, plasma urea, plasma AA, and ADG for their final estimation of the lysine requirement (7.8 g/d lysine). An average of four predictors likely contributed to a more accurate estimation than use of only one predictor.

Although plasma AA concentrations are often used as an indicator for AA requirements, Titgemeyer and Merchen (1990) emphasized that no method has been perfected for use in cattle.

They conducted a nitrogen retention study in which no significant breakpoint in plasma methionine concentrations was observed in response to supplemental methionine. However, breakpoint analysis of nitrogen retention clearly indicated an optimal infusion of 3 g/d L-methionine, and they hypothesized a two-phased response curve may have been evident if supplemental increments had been smaller to allow assessment between 0 and 3 g/d supplemental methionine. This highlights the difficulties associated with estimating AA requirements and underscores the limitations of the frequently used predictors.

The variation in the magnitude of response in plasma amino acid concentrations to supplemental AA is due, in part, to the use of the DL-methionine rather than L-methionine. Campbell et al. (1996) illustrated that both isomers were utilized with similar efficiencies when comparing data for nitrogen retention. However, when comparing plasma methionine concentrations they found the D-isomer led to greater plasma methionine concentrations and hypothesized that the D-isomer may be metabolized more slowly than the L-isomer. Tzeng and Davis (1980) using DL-methionine found unusually large methionine requirements (0.65 g/d BW0.73). Williams and Smith (1974) utilized comparable steers but infused L-methionine and estimated requirements to be much lower (0.25 g/d $BW^{0.73}$). Similarly, Titgemeyer and Merchen (1990) reported plasma methionine concentrations to be significantly greater for cattle infused with 6 g/d DL-methionine when compared to 6 g/d L-methionine. The group attributed the difference to a decrease in rate of uptake and(or) metabolism by the liver of DL-methionine. Clearly, the metabolism of L-methionine differs from that of D-methionine.

Assessment of Limiting Amino Acids

Methionine

Microbial protein often accounts for most of the AA available for absorption at the small intestine (Clark et al., 1992; Mariz et al., 2018) but has been shown to contain relatively low quantities of methionine (Storm and Orskov, 1984; Nimrick et al., 1970); thus, it is reasonable to expect the first limiting AA to be methionine for diets where microbial protein is the predominant AA source for the animal. Although the scope of studies estimating methionine requirements is somewhat limited, an abundance of work suggests methionine is often the first- or second-limiting AA for growing beef cattle.

In a 134-d feeding experiment with 180 crossbred steers, Hosford et al. (2015) examined the effects of ruminally protected methionine with or without the addition of zilpaterol hydrochloride. They found feeding cattle a finishing diet with supplemental methionine and zilpaterol tended to increase ADG (P=0.09) and G:F (P=0.09) when compared to a diet supplemented with only zilpaterol. Similarly, Carvalho et al. (2024) examined the effects of ruminally protected methionine and lysine with or without the addition of ractopamine-hydrochloride in a calf-fed Holstein finishing trial. The group reported an interaction (P=0.04) such that cattle fed the combination of protected AA and ractopamine had greater DMI than cattle fed protected AA or ractopamine alone. They also reported increased carcass weight ($P \leq 0.03$) with protected AA alone. The data suggest protected AA and beta-agonists may have synergistic effects on cattle performance, likely due to an increase in AA requirements for cattle fed beta-agonists.

Cantalapiedra-Hijar et al. (2020) evaluated the effects of ruminally protected methionine supplemented to young bulls fed high-forage diets using a factorial arrangement of two protein

levels (13.5% and 16.2%) and two methionine concentrations (2.0% of MP and 2.6% of MP). Results showcased improved growth performance (P=0.02) when methionine was supplemented at 2.6% of MP compared to 2.0% of MP. The group also reported a trend (P=0.10) for supplemental methionine to have a greater impact in the cattle fed the high protein diet. Reductions in plasma urea in response to supplemental methionine were greater for the high CP diet than the low CP diet, suggesting a non-methionine AA may have been co-limiting in the later diet, and that the limitation was mitigated by increasing protein supply.

Williams and Smith (1974) found methionine to be limiting in Friesian calves fed two diets consisting primarily of straw, flaked corn, groundnut meal, and corn starch. The diets contained 16% groundnut meal or 5% groundnut meal, with the latter diet containing more corn starch. Breakpoint analysis of plasma methionine was similar for both treatments showing a marked increase in concentrations when 4.4 g/d methionine was infused abomassally. This suggests the extent to which methionine was limiting was similar for both groups of calves, despite dietary differences.

A pool of the literature showing methionine to be limiting in ruminants relies on sheep as a metabolic model for cattle. There is little evidence validating this model (Soto-Navarro et al., 2014; Chishti et al., 2019) and caution should be taken when drawing conclusions regarding AA requirements. Although the use of sheep may result in generalizations, the method still offers valuable insights. Foundational work by Chalupa (1975) examined the effects of ruminally protected methionine on nitrogen utilization and plasma concentrations in growing lambs. The lambs were fed a 14% CP diet supplemented with encapsulated methionine; lambs showed the greatest increase in retained nitrogen (6.2 g/d) when 2.0 g/d methionine was supplemented daily. Similarly, plasma methionine remained static until supplemental methionine was increased

beyond 2.0 g/d, suggesting methionine requirements were met at this level of inclusion. These results are consistent with the findings of Schelling et al. (1973) who conducted a series of experiments to examine the effects of supplemental methionine on lambs fed various diets. Despite various inclusions of ground corn and soybean meal, nitrogen retention and plasma AA concentrations invariably showed methionine to be first-limiting and suggested requirements were satisfied by 2.0 to 3.0 g/d supplemental methionine.

As expected, methionine has not been found to be limiting under all experimental conditions. Research conducted by Hussein and Berger (1995) using 168 steers over a 266-d growing and finishing experiment reported no significant change in performance or carcass characteristics in response to ruminally protected methionine or lysine. Two experimental diets contained 71% shelled corn, 10% corn silage, 4% distillers solubles, and 15% protein supplement. The protein supplement brought CP to 13% from only soybean meal or from 50% soybean meal and 50% urea. The methionine and lysine requirements were met by dietary contributions regardless of the protein source. Similarly, Abe et al. (1999) provided growing calves a corn (84%) and soybean meal (14%) based-diet and supplemented with ruminally-protected methionine. For both intake levels provided (20 and 27 g/kg BW), nitrogen retention was not improved with methionine infusion and plasma methionine concentrations exhibited a marked increase after infusion of any amount. This indicates methionine was not limiting under the experimental conditions and suggests methionine requirements are likely to be met by diets consisting primarily of corn. These studies align with the findings from Hill et al. (1980) who provided incremental levels of methionine via abomasal infusion to steers fed a diet based on corn (65%) and cottonseed hulls (30%). Lysine was supplied in excess of requirements to mitigate the chances of obscuring the evaluation of methionine, however nitrogen retention was

not improved by abomasal supplies of methionine, and plasma methionine concentrations exhibited a linear increase as supplemental methionine increased. This further suggests dietary methionine may be provided in sufficient quantities by corn-based diets. It is evident the extent to which methionine is limiting depends on many factors and more comprehensive research is needed concerning the conditions under which supplemental methionine is beneficial.

Lysine

Published literature frequently suggests lysine to be first- or second-limiting, especially when corn-based diets are fed. This is to be expected as a significant portion of corn protein escapes ruminal degradation to become available in the small intestine; additionally corn protein is a relatively poor source of lysine compared to other protein sources (Huang et al., 2005).

Titgemeyer et al. (1988) conducted two experiments to determine the lysine requirements of growing steers. From the first experiment, the group reported that 383 kg steers fed a 75% corn-based diet at 2.0% of BW daily were first limited by lysine. Plasma lysine concentrations exhibited a two-phase broken line response curve, indicative of approaching and then exceeding the animals' requirements as abomasal infusion increased. This suggests requirements were not met via basal flow from microbial and dietary escape protein. The second experiment was conducted with steers fed a diet consisting of 80% corn silage fed at 2.0% of BW daily. The latter experimental diet contained less nitrogen, but neither lysine nor methionine were limiting under these conditions. This is significant as it suggests the silage diet was 1) able to provide sufficient quantities of both lysine and methionine to exceed the animals' requirements, or 2) not able to provide sufficient quantities of other essential AA to the extent they became more limiting.

More recent research conducted by Xue et al. (2011) provided cattle a corn silage and corn grain-based diet over an 84-d growth experiment. Diets were formulated to meet metabolizable protein requirements according to NRC (2000). Incremental inclusion of rumen-protected lysine product up to 15 g/d increased ADG and G:F with diminishing returns noted when more than 10 g/d (3 g/d metabolizable lysine) was provided. Additionally, plasma lysine concentrations remained static until supplemental lysine reached levels of 10 g/d or higher in which case plasma lysine concentrations exhibited a linear increase. Both performance parameters and plasma lysine concentrations suggest that the supplemental lysine was, at least to some extent, preserved from ruminal degradation and lysine was likely the first limiting AA under these experimental conditions.

Histidine

Although most work regarding limiting AA in growing cattle suggest lysine and methionine to be first- and second-limiting, some studies have indicated other AA to be limiting to some extent. Throughout work to identify the limiting AA in microbial protein of sheep, Storm and Ørskov (1984) found histidine, after methionine and lysine, to be limiting. This observation was based on the omission of supplemental histidine leading to decreased nitrogen retention. However, graded omission up to two-thirds of the supplemental histidine did not result in decreased nitrogen retention, suggesting the limitation to be small in scale. It is likely that most practical diets would have supplied sufficient histidine to meet requirements beyond of microbial supply.

Veira et al. (1988) also found histidine to be limiting through examination of the AA proportions in duodenal digesta of growing calves. The group reported 0.29 g histidine per 1 g lysine in duodenal digesta compared to 0.39 g histidine per 1 g lysine found in calf carcass.

Because this silage-based diet was found to be lysine deficient, it was also unlikely to provide a sufficient quantity of histidine. These results should be interpreted cautiously as some AA have functions other than tissue growth.

Wessels et al. (1997) found that histidine may have been first limiting for steers fed a diet consisting primarily of 85.1% rolled corn, 10.4% prairie hay, and 1.1% urea. This was based upon the concept that the AA showing the smallest increase in plasma concentration relative to the quantity supplied post-ruminally is likely the first-limiting AA. Plasma histidine concentrations (91.6 to 91.9 μM) remained static relative to the quantity of supplemental histidine. Had histidine supply surpassed animal requirements, a sharp increase in plasma concentrations would have been noted. As corn is notably limiting in lysine and microbial protein may be lacking in methionine, histidine is a surprising suspect for a first-limiting AA.

Greenwood and Titgemeyer (2000) illustrated that histidine was limiting in steers (152 kg) fed a diet consisting of 72% soybean hulls, 19% alfalfa, and 5% molasses. In addition to a diet low in ruminally degradable protein (RUP), steers were provided a mix of ten essential AA through abomasal infusion; removal of histidine from the mixture resulted in a significant decrease in nitrogen retention ($P<0.05$). As decreased supply of a limiting AA is expected to decrease nitrogen retention, the data suggest diets low in RUP may not supply sufficient quantities of histidine. However, in this study methionine was identified as the first-limiting AA.

Threonine

Through methods similar to those mentioned previously, Wessels et al. (1997) predicted threonine to be limiting for steers fed a corn-urea diet as plasma concentrations did not exhibit a sharp increase as supplemental threonine was increased. The static plasma concentrations of both histidine and threonine in response to abomasal infusion may suggest a co-limitation under these

conditions. Under conditions satisfying lysine and methionine requirements, Richardson and Hatfield (1978) reported a significant increase in nitrogen retention with supplemental threonine. Furthermore, they observed no significant increase in nitrogen retention with the addition of histidine and tryptophan to the threonine treatment suggesting that threonine was the third-limiting amino acid, following methionine and lysine.

In a foundational study utilizing lambs, Nimrick et al. (1970) fed a semi-purified diet consisting of cornstarch (57%), cellulose (25%), molasses (5%), urea (4.3%), and corn oil (3%). The group reported threonine to be limiting, at least to a minor extent. This conclusion was based on the observed decrease in plasma threonine concentrations when methionine was supplied in excess, suggesting threonine was being utilized in greater quantities than were supplied. Furthermore, supplemental threonine only improved nitrogen retention when both methionine and lysine were provided in excess. Although threonine may not be first limiting under most experimental conditions, the data suggests it may become limiting once the requirements for methionine and lysine are satisfied.

Tryptophan

Although there is limited data illustrating conditions in which tryptophan is a limiting AA, it cannot be completely ruled out. Abe et al. (1998) conducted a study with freshly weaned calves fed a diet based on corn and soybean meal and reported methionine and lysine to be first- and second-limiting. However, they also reported tryptophan to possibly be co-limiting or third-limiting; nitrogen retention was increased more than 2 g/d with tryptophan supplementation when compared to methionine alone. In contrast, Storm and Ørskov (1984) found no decrease in nitrogen retention with the omission of supplemental tryptophan from an essential AA mix, indicating tryptophan was not limiting in lambs receiving microbial protein as the only source of

AA. It is unlikely tryptophan will become a major limiting nutrient under most ruminant production conditions.

Arginine

Storm and Ørskov (1984) reported decreased nitrogen retention for lambs when arginine was omitted from the essential AA mix. Similar to the effects of removing histidine, no decrease was observed until at least two thirds of the supplemental arginine was removed. This would suggest the arginine requirement was met with relatively small additions to microbial protein. Additionally, Veira et al. (1988) compared the AA composition of calf carcasses to the AA composition of duodenal digesta in calves fed grass silage-based diets. They found the lysine to arginine ratio in carcass lean to be 1.03 g/g whereas the ratio in digesta was 0.66 g/g. Because the digesta contained greater proportions of arginine than lean tissue, it is unlikely arginine would become limiting before lysine. These findings suggest arginine requirements are relatively modest and are likely to be met through microbial contributions.

Conclusion

Methionine and lysine are often the first-limiting AA for protein accretion in beef cattle. Methodologies to predict AA requirements have been inconsistent, resulting in variable estimates of requirements. Data suggest other essential AA are situationally limiting or co-limiting dependent upon diet composition, intake, and performance. Despite inconsistency among attempts to identify and quantify the extent of these limitations, it is advantageous to consider each AA individually in the context of protein supplementation; there is no single, all-encompassing solution.

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Chapter II – Effect of post-ruminal lysine, methionine, and 10 essential amino acids on nitrogen utilization and plasma amino acid profile of growing steers fed a corn-based diet

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Abstract

Growing cattle rely on microbial protein to meet much of their amino acid (AA) requirements. However, optimal growth and performance may require AA supplies beyond microbial contribution. Ensuring adequate post-ruminal, absorbable AA is therefore essential for achieving peak productivity. Seven ruminally cannulated Angus × Holstein cross steers (191.6 ± 12.9 kg) were used in a 6 × 6 Latin square to determine which AA were limiting for cattle fed corn-based diets. Six treatments were infused abomasally across 6-d periods and included: 1) control (water infusion); 2) 6 g/d lysine (6Lys); 3) 2 g/d methionine (2Met); 4) 6 g/d lysine plus 2 g/d methionine (6Lys2Met); 5) 12 g/d lysine plus 4 g/d methionine (12Lys4Met); 6) 12 g/d lysine, 4 g/d each of methionine, histidine, leucine, isoleucine, valine, threonine, arginine, phenylalanine, and 1 g/d tryptophan (MIX). Steers were fed 4.5 kg/d (dry matter basis) of diet based on corn and corn gluten feed. Periods included 2-d adaptations and 4 d for total fecal and urine collections to measure nitrogen retention. Blood samples were collected on d 6 and analyzed for plasma AA concentrations. Data were analyzed as a Latin square with fixed effects of treatment and period and random effect of steer. Means were separated with pairwise t-tests. Intake and digestibility of organic matter were not affected by treatment ($P>0.25$). Compared to nitrogen retention of control (26.0 g/d), MIX (33.2 g/d; $P<0.01$), 6Lys2Met (29.5 g/d, $P=0.01$), and 12Lys4Met (29.3 g/d; $P=0.02$) increased nitrogen retention. Because 6Lys2Met and 12Lys4Met yielded similar improvements in nitrogen retention, supplemental requirements were not greater than 6 g/d Lys and 2 g/d Met when no other amino acids were provided. The greater response for MIX than for 12Lys4Met ($P=0.01$) demonstrates another essential AA became limiting once supplemental requirements for Lys and Met were met. The failure of 6Lys ($P=0.14$) and 2Met ($P=0.59$) to improve nitrogen retention suggests that Lys and Met were

colimiting. Plasma Lys concentrations were greater (P<0.05) than control (73.8 μM) for 12Lys4Met (111.2 μM) and MIX (104.6 μM). Lys requirements were likely met somewhere between 6 and 12 g/d as increases in plasma Lys were much greater when post-ruminal Lys increased from 6 to 12 g/d than when post-ruminal Lys increased from 0 to 6 g/d. Plasma Met was greater (P<0.05) for 2Met (34.0 μM) than for control (29.0 μM); however, plasma Met for 6Lys2Met (31.2 μM) was not different (P>0.10) than control, suggesting greater utilization of Met for 6Lys2Met than for 2Met. Utilization efficiency of Lys and Met was greater for 6Lys2Met and MIX than for 6Lys or 2Met alone. Efficiency was lower for 12Lys4Met than for 6Lys2Met, indicating supplemental Lys and Met requirements had been exceeded at the highest supplementation amounts. The data suggest Lys and Met were co-limiting for growing cattle fed a corn-based diet, and at least one additional essential AA was also limiting.

Introduction

Maximizing cattle performance depends on meeting their amino acid (AA) requirements. Microbial protein, which is the primary source of AA for ruminants, along with basal supplies of ruminally undegraded dietary protein often exceed requirements. Yet, ambitious production goals may increase AA demands beyond that of basal supplies. Consequently, conditions may warrant provision of post-ruminally absorbable AA to growing cattle to facilitate optimal performance.

Of the essential AA, lysine (Lys) and methionine (Met) are frequently reported first-limiting for growing cattle (Richardson and Hatfield, 1978; Klemesrud et al., 2000). The limitation for Met is often attributed to its low concentrations in microbial protein (Storm and Ørskov, 1984), whereas the Lys limitation is often attributed to the low concentrations found in corn and corn-based products (Shewry, 2007). Multiple attempts to predict requirements of Lys and Met in growing cattle have resulted in variable outcomes. Data suggest other essential AA are situationally limiting or co-limiting dependent upon diet composition, intake, and performance (Richardson and Hatfield, 1978; Storm and Ørskov, 1984; Wessels et al., 1997). Individual AA requirements and the factors affecting them remain unclear.

The objective of this experiment was to identify the limiting AA for growing steers fed a corn-based diet and provide insight to the extent of these limitations.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved all procedures involving the use of animals (IACUC-4902).

Animals and Experimental Treatments

Seven Angus × Holstein steers (BW at trial initiation = 192 ± 13 kg) were obtained from western Kansas. Upon arrival they were placed in tie stalls within a temperature-controlled room (16 °C) that provided 16 h of light daily. Steers were fed 3.6 kg/d (dry matter basis) of the corn-based experimental diet (Table 2.1) and given 2 weeks to adapt before ruminal cannulation surgery. Steers were allowed 1 month recovery after ruminal cannulation before starting the experiment. During the final week of recovery, steers were started on final diet intake of 4.5 kg/d (dry matter basis) fed in two equal portions at 12 h intervals. After adaptation to increased diet intake, steers were placed in metabolism crates and tygon tubing (2.38 mm i.d.; Fisher Scientific, Pittsburgh, PA) was placed through the ruminal cannula of each steer and directed through the reticulo-omasal orifice and into the abomasum. These lines were held in the abomasum with a 9-cm rubber flange. Steers were given 8 d of adaptation to metabolism crates, with water infusions into the abomasum the last 2 d before the start of the experiment. Treatments were infused from bottles (changed at 12 h intervals) via peristaltic pump (Ismatec ISM444A-230V BVP Standard; Cole-Parmer Instrument Company, Vernon Hills, IL) at a rate of 4 kg/d.

Within the 6 × 6 Latin square, treatment sequences were balanced for carryover effects and the additional steer received the same treatment sequence as another steer throughout the experiment. Periods were 6 d in length, including 2 d for adaptation and 4 d for total collection of urine and feces, with blood collection on d 6. All treatments were infused abomasally and included: 1) control (no AA, water infusion); 2) 6 g/d lysine (6Lys); 3) 2 g/d methionine (2Met);

4) 6 g/d lysine and 2 g/d methionine (6Lys2Met); 5) 12 g/d lysine and 4 g/d methionine (12Lys4Met); 6) 12 g/d lysine, 4 g/d methionine, 4 g/d histidine, 4 g/d leucine, 4 g/d isoleucine, 4 g/d threonine, 4 g/d arginine, 4 g/d phenylalanine, 4 g/d valine, and 1 g/d tryptophan (MIX). All infused AA were L-isomers.

A Lys treatment solution was prepared by dissolving 122.2 g L-lysine-HCl (96 g L-lysine) in 3,077.8 g water. A Met treatment solution was prepared by dissolving 32.0 g L-methionine into 3168.0 g water. Essential AA treatment solution was prepared by dissolving 8 g L-threonine, 8 g L-phenylalanine, 8 g L-leucine, 8 g L-valine, 8 g L-isoleucine, 8 g L-arginine, 2 g L-tryptophan, and 10.8 g L-histidine-HCl-H_2O (8 g L-histidine) in 3931.2 g water. The treatment solutions were refrigerated from mixing until use. Infusion bottles (2 per steer per day, each providing for 12 h of infusion) were prepared by adding treatment solutions and water to a total weight of 2 kg. For steers receiving 6 g/d Lys, 100 g Lys solution was added to each bottle. For steers receiving 12 g/d Lys, 200 g Lys solution was added to each bottle. In like manner, for steers receiving 2 g/d Met, 100 g Met solution was added to each bottle. For steers receiving 4 g/d Met, 200 g Met solution was added to each bottle. Lastly, for steers receiving the 8 remaining essential AA, 1000 g essential AA solution was added to each bottle.

Sample Collection

Feed ingredients were sampled individually prior to diet mixing and stored at –20 °C. Representative diet samples from every period were collected at each feeding on d 2 to 5 and stored at –20 °C. Diet samples were composited within period then subsampled and stored at –20 °C. Orts, if present, from d 2 through 5 were collected and weighed, composited by steer within each period, and then subsampled and stored at –20 °C. Urine and feces from d 3 through 6 were collected and weighed daily. Urine was collected into buckets containing 900 mL of 10% (wt/wt)

H_2SO_4 for ammonia preservation. Feces were collected in pans lined with plastic bags to facilitate mixing. Representative samples of urine and feces were obtained from the daily samples and stored at –20 °C. Orts, feces, and urine were composited by steer within each period; composites were created as proportions of total outputs and stored at –20 °C.

Jugular blood samples were collected on d 6 of each period at 4 h after feeding into 7.5 mL sodium heparin tubes (SARSTEDT Inc., Newton, NC). Tubes were immediately inverted, placed on ice, and centrifuged at 1,200 × g at 4 °C for 20 min. The supernatant was transferred to a 2-mL microcentrifuge tube and stored at –20 °C.

Laboratory Analyses

Diet and orts samples were dried in a 55 °C forced-air oven for 48 h, allowed to air-equilibrate, weighed, and then ground through a 1-mm screen with a Thomas Wiley Mill (Thomas Scientific, Swedesboro, NJ). Samples were dried in a 105 °C forced-air oven for 24 h to determine total dry matter (DM), followed by 12 h at 450 °C in a muffle oven to determine organic matter (OM) content. Wet fecal samples were dried in a 105 °C forced-air oven for 24 h, followed by 12 h in a 450 °C muffle oven for determining total DM and OM contents. Nitrogen content was measured in duplicate for diet, orts, wet feces, and urine with a LECO Nitrogen Analyzer (LECO Corporation, Saint Joseph, MI).

Plasma samples (0.75 mL) were deproteinized prior to amino acid analysis by mixing plasma with an equal volume of 10% (wt/vol) sulfosalicylic acid containing 600 µM norvaline as an internal standard. Samples were vortexed and placed on ice for 1 h, with recurring agitation every 15 min. Samples were then centrifuged at 17,000 × *g* for 20 min at 4 °C, the supernatant was filtered through a 0.2-micron syringe filter (Thermo Fisher Scientific Inc., Waltham, MA), and then stored at –20 °C before thawing and derivatizing using 6-aminoquinolyl-N-

hydroxysuccinimidyl carbamate (AccQ-Tag Ultra Derivatization Kit; Waters Corporation, Milford, MA). Derivatized samples were analyzed using ultra-high pressure liquid chromatography (Waters Acquity UPLC; Waters Corporation, Milford, MA). Samples were separated using a C18 column and detected with a spectrophotometer at 260 nm.

Calculations

Digestibilities of DM and OM were calculated with intake solely from dietary origin. Retained nitrogen was calculated as the difference between nitrogen intake (intake + infused) and nitrogen output (urinary + fecal). AA utilization efficiencies were calculated as the increase in AA deposition divided by the increase in supplemental AA supply. Whole carcass AA compositions (g tissue AA/g tissue protein) were based on Ainslie et al. (1993).

Amino Acid Utilization Efficiency

$$\frac{(\text{Treatment Retained N, g/d} - \text{Control Retained N, g/d}) \times \frac{6.25\text{ g Protein}}{1\text{ g N}} \times \frac{\text{Tissue AA, g}}{\text{Tissue Protein, g}}}{\text{Supplemental AA, g/d}}$$

Data and Statistical Analysis

The experiment was designed to provide seven observations per treatment; however, one observation for MIX was omitted from statistical analysis due to failure of treatment infusion. Data were analyzed as a Latin square with fixed effects of treatment and period and random effect of steer using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Treatment means were derived from LSMEANS statement and separated with pairwise t-tests. Significance and tendencies were established at $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively.

Results and Discussion

Nitrogen Retention

If an AA is the first limiting nutrient for protein deposition, supplementation of that AA is expected to increase nitrogen retention. In this way nitrogen retention can serve as a reliable indicator for identifying limiting AA under specific conditions and can provide insight into the extent of its limitation. Nitrogen retention represents the difference between nitrogen intake and excreted nitrogen; neither urinary (P=0.71) or fecal (P=0.07) excretion of nitrogen were affected by treatment. Thus, changes in nitrogen retention were largely a result of changes in total nitrogen intake (P<0.01). However, as feed nitrogen (P=0.29) was not affected by treatment, changes in nitrogen retention were a direct result of additional nitrogen provided through infusion. Although the magnitude of change in nitrogen retention appears to be similar to the magnitude of change in infused nitrogen, it is crucial to recognize nitrogen retention is a reflection of all AA incorporated into deposited protein, not solely those provided by infusion. Nitrogen retention (Table 2.2) was greater for MIX (33.1 g/d, P<0.001), 6Lys2Met (29.5 g/d, P=0.01), and 12Lys4Met (29.3 g/d, P=0.02) than control (26.0 g/d). Richardson and Hatfield (1978) observed a similar trend in steers reliant solely on protein of microbial origin as abomasal infusion of Lys and Met significantly increased nitrogen retention. Additionally, Richardson and Hatfield (1978) reported the greatest increase in nitrogen retention when Lys and Met were supplied in combination compared to Met alone. Similarly, we observed no increase in nitrogen retention in response to individual supplementation of Lys (P=0.14) or Met (P=0.59). This lack of response, paired with increased nitrogen retention when Lys and Met were supplied together, suggests these AA are co-limiting for growing cattle fed corn-based diets.

Intake and Digestibility

Steers were limit-fed approximately 2.3% of BW daily on a DM basis throughout the experiment to minimize refusals and thereby reduce variation. Intake of DM averaged 98.4% of DM offered; as expected DM and OM intakes did not differ among treatments (P=0.25, P=0.29, respectively). Digestibility of DM and OM were not affected by treatments (P=0.50, P=0.39, respectively). Similar observations were reported by Campbell et al. (1997) as incremental amounts of abomasally infused methionine to growing steers did not have an impact on DM or OM digestibility. Additionally, Greenwood and Titgemeyer (2000) reported no changes in DM or OM digestibility when Met or essential AA were supplied post-ruminally, or when Lys, threonine, histidine, phenylalanine, tryptophan, branched-chain AA, or arginine were removed individually from post-ruminal infusion. This suggests changes in nitrogen retention associated with post-ruminal AA supplementation are not likely attributable to changes in dietary intake or nutrient absorption but rather improved nitrogen utilization as AA requirements are met.

Plasma Concentrations

Lysine

Plasma AA concentrations are presented in Table 2.3. Lys concentrations were not affected by post-ruminal supply of 2Met or 6Lys individually, nor were they affected by infusion of their combination when compared to control (P>0.10). However, plasma Lys was found to be greater (P<0.05) than control (73.8 μM) for 12Lys4Met (111.2 μM) and MIX (104.6 μM). The nonsignificant and proportionately smaller increases in plasma Lys concentrations when post-ruminal supplies of Lys were increased from 0 to 6 g/d followed by larger increases when amounts were increased from 6 to 12 g/d lysine suggest the steers' requirements were satisfied at some point between 6 and 12 g/d, with 12 g/d Lys exceeding metabolic needs. This conclusion is based on the suggestion that with increasing post-ruminal infusion, the plasma concentrations of

a limiting AA usually remain static until the requirement is met, at which point they begin to increase (Burris et al., 1976). This aligns with our observations of nitrogen retention not increasing when Lys supplementation was increased from 6 to 12 g/d in combination with Met. However, it is possible supplemental Lys requirements were greater than 6 g/d when MIX was supplemented and limitations of at least some of the other AA were eliminated or reduced. Titgemeyer et al. (1988) assessed the Lys requirements of growing steers fed corn-based diets and reported similar findings when infusing increasing supplies of post-ruminal Lys with or without Met. In both cases, plasma Lys exhibited a two-phase broken line in which the regression accounting for approximately 70% of the variation in the data; however, in that work, no direct measures of protein deposition were available to verify that requirements based on plasma Lys concentrations were valid. Batista et al. (2016) provided increasing supplies of post-ruminal Lys and observed a clear discrepancy between the breakpoint analysis based on nitrogen retention (supplemental Lys requirement = 9 g/d) and that derived from plasma concentration (supplemental Lys requirement = 3 g/d).

Methionine

As with plasma Lys, plasma Met concentration has been reported to increase when post-ruminal Met supply is increased (Williams and Smith, 1974). In our experiment plasma Met was greater for 2Met (34.0 μM) than for control (29.0 μM); however, plasma Met for 6Lys2Met (31.2 μM) was not different than control. This is likely due to Lys supplementation increasing the utilization of Met for protein deposition and thereby decreasing plasma Met concentrations; this supports the suggestion that Lys and Met were at least partially co-limiting. Although not statistically significant, the co-limiting nature of Lys and Met is supported by observations that plasma Lys concentrations were numerically (P=0.33) less for 6Lys2Met than for 6Lys. Plasma

Met concentrations were greater when larger quantities were infused; 4 g/d methionine provided as 12Lys4Met or MIX increased plasma Met concentrations ($P<0.01$) to 43.9 and 39.2 μM, respectively. Although not significant ($P=0.09$), the numerically lesser Met concentration associated with MIX than with 12Lys4Met further demonstrates that supplying additional essential AA likely increases the utilization of methionine for protein deposition. This interpretation is consistent with nitrogen balance data where MIX yielded a greater nitrogen retention than 12Lys4Met. Plasma Met was increased with 2Met ($P=0.04$), yet nitrogen retention was not, suggesting Met was not first- and solely-limiting. Yet, 6Lys2Met increased nitrogen retention ($P=0.01$) without affecting plasma Met concentrations, leading to the conclusion that Met was a co-limiting AA when Lys was supplemented.

Essential Amino Acids Besides Lysine and Methionine

Theoretically, post-ruminal supplementation of limiting AA is expected to increase the utilization of the remaining AA until another nutrient becomes limiting or the animal's biological potential to deposit protein is met. Xue et al. (2011) demonstrated this as they supplied incremental increases of ruminally protected Lys to growing bulls. As Lys supplementation increased, Met and threonine plasma concentrations were significantly decreased. Abe et al. (1998) reported similar results when supplementing Lys and Met to young calves; plasma concentrations for the branched-chain AA decreased significantly with post-ruminal Lys and Met, whereas plasma concentrations of threonine, phenylalanine, and histidine decreased numerically. In our experiment, histidine, tryptophan, and valine ($P<0.05$) plasma concentrations were decreased by 6Lys2Met and 12Lys4Met. Additionally, plasma concentrations for most essential AA were increased by MIX. This was expected because increased post-ruminal supply elevates plasma concentrations, especially when the requirements for those AA are satisfied.

Thus, we could conclude that, although at least some of the additional 8 essential AA added to MIX were deficient when steers received 12Lys4Met, it appears that supplies of many of the essential AA were above the requirements when MIX was supplemented. The inability of supplemental tryptophan in MIX to raise plasma tryptophan concentrations above those in control might suggest that it became a limiting AA when MIX was provided to the steers.

Non-Essential Amino Acids

Due to the complex nature of AA metabolism, varying results have been reported with regards to NEAA plasma concentrations in response to post-ruminal EAA supplementation. Hill et al. (1980) provided post-ruminal infusions of lysine and methionine to steers fed primarily corn and cottonseed hulls with 1.2% inclusion of urea. The group reported no changes in overall non-essential AA totals with slight decreases in glutamic acid as Lys and Met supply were increased; the authors attributed the decreased plasma glutamate concentration to improved AA status and assumed changes were of little physiological importance. Similarly, 6Lys2Met resulted in decreased glutamate ($P<0.05$), although the effect was not observed for 12Lys4Met ($P>0.10$). We also observed a decrease in plasma taurine concentration with 6Lys. This may be the result of greater utilization of methionine for protein deposition, which would result in less cysteine production and consequently less taurine production (Stipanuk et al., 2010).

Amino Acid Utilization Efficiency

Lysine

The efficiency in which supplemental AA are utilized for protein deposition can provide insight into the extent to which these AA are limiting (Table 2.4). As 6Lys2Met (22%) resulted in greater utilization efficiency of Lys than 6Lys (13%), Lys and Met were likely co-limiting under the conditions of this experiment. The data suggests supplemental Lys requirements were exceeded with 12Lys4Met, as it resulted in lower utilization efficiency of Lys (10%) than 6Lys2Met. This is attributable to the fact that increasing the supply of a supplemental AA beyond its requirement will result in a decrease in its efficiency of use for protein deposition (Batista et al., 2016). Additionally, utilization efficiency of Lys for MIX (22%) was the same as 6Lys2Met, indicating supplementation of at least one EAA besides Lys and Met contributed to a greater capacity for Lys use in protein deposition than 12Lys4Met. The efficiency of Lys utilization reported by Batista et al. (2016) was notably greater (40%) than observed in the current experiment, despite using a similar approach with post-ruminal infusion of 6 g/d Lys to Holstein steers. While Lys appeared limiting in both experiments, it is possible efficiency was lower for the current experiment due to 6 g/d supplemental Lys exceeding requirements.

Methionine

Observations for Met utilization efficiency were similar to those for Lys. First, 6Lys2Met (22%) contributed to greater utilization efficiency of Met than 2Met (5%) alone, further suggesting Lys and Met were co-limiting under experimental conditions. Additionally, utilization efficiency of Met was lower for 12Lys4Met (11%) than 6Lys2Met. This indicates supplemental Met was provided in excess of requirements at 4 g/d, at least until other limiting EAA, apart from Lys, were provided. MIX demonstrated the same utilization efficiency of Met (22%) as 6Lys2Met, further demonstrating supplementation of at least one EAA besides Lys and Met

satisfied a limitation and allowed for greater use of Met for protein deposition. The Met utilization efficiency for 6Lys2Met and MIX were similar to the 24% efficiency demonstrated by Campbell et al. (1997). However, Löest et al. (2002) reported a Met utilization efficiency of 43% when abomasally infusing Met into steers under experimental conditions designed to limit Met supply. Additionally, Lambert et al. (2004) reported Met utilization efficiencies of 27 and 63% for steers under Met-limiting conditions, with the greater efficiency attributed to abomasal infusion of glycine. As a whole, those studies demonstrate variability in Met utilization efficiency.

Conclusions

Lys and Met were co-limiting for growing steers fed our corn-based diet with supplemental requirements no greater than 6 g/d Lys and 2 g/d Met when no other AA were supplemented. Additionally, once Lys and Met requirements were satisfied, at least one other essential AA became limiting. Diets primarily comprised of corn and corn-based products fed to growing steers may warrant supplemental Lys and/or Met. Further investigation regarding the extent of AA limitations under various production settings will be required before effective supplementation strategies can be widely implemented to optimize performance.

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Table 2.1. Diet composition

Item	% of DM
Ingredient	
Dry-rolled corn	55.7
Dried corn gluten feed	22.6
Prairie hay	16.1
Molasses	3.1
Limestone	2.0
Trace mineral salt[1]	0.4
Vitamin and mineral premix[2]	0.1
Composition	
DM, % as fed	90.5
OM	92.0
CP	9.8

[1]Composition (at minimum): 96.0% NaCl, 0.24% Mn, 0.24% Fe, 0.032% Zn, 0.026% Cu, 0.007% I, and 0.004% Co

[2]Provided (per kg diet DM): 1800 IU vitamin A, 200 IU vitamin D, 20 IU vitamin E, 26.48 mg Zn, 8.83 mg Cu, 8.83 mg Mn, and 0.18 mg Se

Table 2.2. Effects of post-ruminal lysine, methionine, lysine plus methionine, or a mixture of 10 essential amino acids on nitrogen utilization of steers fed a corn-based diet

	Treatment[1]							
Item[2]	Control	2Met	6Lys	6Lys2Met	12Lys4Met	MIX	SEM[3]	Trt *P*
n=	7	7	7	7	7	6		
DMI, kg/d	4.44	4.45	4.41	4.45	4.35	4.45	0.07	0.25
OMI, kg/d	4.09	4.10	4.07	4.15	4.01	4.10	0.06	0.26
DMD, g/g	0.725	0.712	0.726	0.736	0.731	0.716	0.011	0.50
OMD, g/g	0.746	0.731	0.748	0.758	0.756	0.742	0.012	0.39
Nitrogen, g/d								
Feed	72.1	72.3	71.8	73.1	70.7	72.2	1.0	0.29
Infused	0.0	0.2	1.1	1.3	2.7	7.3	-	-
Total intake	72.1[a]	72.5[a]	72.9[ab]	74.4[b]	73.4[ab]	79.5[c]	1.0	<0.001
Fecal	29.5	30.2	28.1	28.1	27.5	29.8	0.8	0.07
Urine	16.7	15.6	16.8	16.8	16.6	16.7	1.6	0.71
Retained	26.0[a]	26.7[ab]	28.0[ab]	29.5[b]	29.3[b]	33.2[c]	1.6	<0.001
Nitrogen, % of total intake								
Fecal	40.8[a]	41.6[a]	38.5[ab]	37.8[b]	37.3[b]	37.4[b]	0.9	0.004
Urine	23.1	21.5	23.0	22.6	22.7	21.0	2.1	0.53
Retained	36.0[a]	36.8[a]	38.4[a]	39.6[b]	39.9[b]	41.6[b]	2.1	0.02

[1]Control: water; 2Met = 2 g/d methionine; 6Lys = 6 g/d lysine; 6Lys2Met = 6 g/d lysine plus 2 g/d methionine; 12Lys4Met = 12 g/d lysine plus 4 g/d methionine; MIX = 12 g/d lysine, 4 g/d methionine, 4 g/d histidine, 4 g/d leucine, 4 g/d isoleucine, 4 g/d valine, 4 g/d threonine, 4 g/d arginine, 4 g/d phenylalanine, 4 g/d valine, and 1 g/d tryptophan; SEM = standard error of mean.
[2]DMI = dry matter intake; OMI = organic matter intake; DMD = dry matter digestibility; OMD = organic matter digestibility.
[3]SEM = standard error of mean.
[a,b,c] Means within row not bearing a common superscript letter differ, $P<0.05$.

Table 2.3. Effects of post-ruminal lysine, methionine, lysine plus methionine, or a mixture of 10 essential amino acids on plasma amino acid concentrations of steers fed a corn-based diet

Item	Treatment[1]						SEM	Trt *P*
	Control	2Met	6Lys	6Lys2Met	12Lys4Met	MIX		
n=	7	7	7	7	7	6		
Lysine	73.8	69.3	84.7	75.6	111.2[A]	104.6[A]	8.0	<0.001
Methionine	29.0	34.0[A]	27.7	31.2	43.9[A]	39.2[A]	2.6	<0.001
Histidine	61.2	63.6	54.1	50.6[A]	49.0[A]	69.1[A]	4.3	<0.001
Tryptophan	31.1	30.6	27.1	26.3[A]	21.5[A]	29.0	2.4	0.003
Threonine	81.2	80.8	67.1[A]	62.3[A]	59.2[A]	97.9[A]	6.5	<0.001
Leucine	111.6	115.2	120.4	104.9	111.0	126.4	7.5	0.27
Isoleucine	79.1	80.7	80.4	72.6	72.6	95.0[A]	5.8	0.05
Valine	165.0	162.1	161.3	138.4[A]	146.0[A]	193.7[A]	7.9	<0.001
Phenylalanine	54.0	57.0	56.0	53.0	53.1	64.3[A]	3.4	0.08
Arginine	97.2	95.4	90.3	87.2	92.9	106.9	5.8	0.15
Alanine	139.9	146.2	146.6	154.0	166.4	157.5	12.6	0.24
Asparagine	32.8	35.8	33.6	32.4	30.1[A]	31.9	2.6	0.50
Aspartate	3.8	4.9	4.3	4.1	4.5	5.6	0.9	0.52
Glutamate	160.2	162.0	150.2	145.4[A]	166.2	160.1	5.2	0.03
Glutamine	320.8	314.5	310.4	298.8	310.3	312.6	14.8	0.83
Glycine	221.3	194.2[A]	203.5	196.1	180.6[A]	195.3	14.4	0.10
Ornithine	50.2	51.5	41.2	39.9[A]	48.9	54.9	3.7	0.07
Proline	61.8	61.4	63.3	60.1	61.1	61.8	3.7	0.98
Serine	67.7	63.7	66.4	63.8	60.2	71.9	4.7	0.52
Taurine	40.1	44.3	33.5[A]	37.0	44.3	37.0	2.5	0.004
Tyrosine	87.0	85.7	83.7	80.8	74.8[A]	82.0	3.4	0.07
Total	1976.7	1952.6	1926.2	1827.3	1863.0	2086.8	79.5	0.21

[1]Control = water; 2Met = 2 g/d methionine; 6Lys = 6 g/d lysine; 6Lys2Met = 6 g/d lysine plus 2 g/d methionine; 12Lys4Met = 12 g/d lysine plus 4 g/d methionine; MIX = 12 g/d lysine, 4 g/d methionine, 4 g/d histidine, 4 g/d leucine, 4 g/d isoleucine, 4 g/d valine, 4 g/d threonine, 4 g/d arginine, 4 g/d phenylalanine, 4 g/d valine, and 1 g/d tryptophan; SEM = standard error of mean; superscripts represent comparison to Control [A] = $P \leq 0.05$.

Table 2.4. Effects of post-ruminal lysine, methionine, lysine plus methionine, or a mixture of 10 essential amino acids on amino acid utilization efficiency of steers fed a corn-based diet

	Treatment[1]				
Item[2]	2Met	6Lys	6Lys2Met	12Lys4Met	MIX
Lysine	-[3]	13	22	10	22
Methionine	5	-	22	11	22
Histidine	-	-	-	-	23
Tryptophan	-	-	-	-	25
Threonine	-	-	-	-	39
Leucine	-	-	-	-	64
Isoleucine	-	-	-	-	25
Valine	-	-	-	-	37
Phenylalanine	-	-	-	-	34
Arginine	-	-	-	-	66

[1]2Met = 2 g/d methionine; 6Lys = 6 g/d lysine; 6Lys2Met = 6 g/d lysine plus 2 g/d methionine; 12Lys4Met = 12 g/d lysine plus 4 g/d methionine; MIX = 12 g/d lysine, 4 g/d methionine, 4 g/d histidine, 4 g/d leucine, 4 g/d isoleucine, 4 g/d valine, 4 g/d threonine, 4 g/d arginine, 4 g/d phenylalanine, 4 g/d valine, and 1 g/d tryptophan.
[2]Data for each item presented as supplemental AA used for protein deposition relative to total supplemental AA supplied, %.
[3]Efficiencies were not calculated for AA not provided by treatments.

Chapter III - Effect of deleting amino acids from a post-ruminally provided essential amino acid mixture on nitrogen utilization and plasma amino acid profile of growing steers fed a corn-based diet

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Abstract

Providing growing cattle with adequate quantities of metabolizable amino acids (AA) is essential for optimal growth and performance. Six ruminally cannulated Angus × Holstein cross steers (238 kg) were used in a 6 × 6 Latin square to determine which AA were limiting for cattle fed corn-based diets. Six treatments were infused abomasally across 6-d periods and included: 1) 12 g/d lysine plus 4 g/d each of methionine, histidine, leucine, isoleucine, valine, threonine, arginine, phenylalanine, and 1 g/d tryptophan (MIX); 2) MIX without lysine (–Lys); 3) MIX without methionine (–Met); 4) MIX without histidine (–His); 5) MIX without leucine, isoleucine, and valine (–BCAA); 6) MIX without threonine and tryptophan (–Thr/Trp). Steers were fed 5.3 kg/d (DM basis) of a diet based on corn and corn gluten feed. Periods included 2-d adaptations and 4 d for total fecal and urine collections to measure nitrogen retention. Blood samples were collected on d 6 and analyzed for plasma AA concentrations. Data were analyzed as a Latin square with fixed effects of treatment and period and random effect of steer. Means were separated with pairwise t-tests. Removal of individual AA from MIX resulted in, at least numerically, decreased plasma concentration of the removed AA. Plasma concentrations for most non-lysine and non-methionine AA were numerically greater for –Lys and –Met than for MIX. Nitrogen retention (42.2 g/d) was numerically greatest for steers receiving MIX. Nitrogen retention tended to be lower for –Lys (35.1 g/d; P=0.11) and –Met (35.6 g/d; P=0.11) than MIX. Nitrogen retention was not significantly altered for –Trp/Thr (36.6 g/d; P=0.17), –BCAA (39.7 g/d; P=0.51), or –His (40.5 g/d; P=0.72). The data indicates lysine and methionine are first- and co-limiting essential AA for growing cattle fed a corn-based diet.

Introduction

Optimizing protein provision and utilization in growing cattle inherently improves production efficiency and can reduce environmental impacts associated with over-feeding protein. Historically, this task has been approached by estimating and meeting or exceeding crude protein or metabolizable protein requirements. Under this nutritional approach, individual amino acids (AA) and their utilization efficiencies are not considered, thus requirements may not be met or wasteful amounts of AA may be fed. Predicting individual AA requirements offers substantial advantages over this system. However, such a system has remained elusive, with attempts to establish a reliable approach leading to contradictory results. As an example, Williams and Hewitt (1979) estimated supplemental lysine requirements for pre-ruminant calves using three markers: plasma AA concentrations, nitrogen retention, and plasma urea concentrations. They reported lysine requirements ranging from 7.2 to 8.5 g/d. Contradictory estimates of total methionine requirements for growing steers of similar size and growth rate by Tzeng and Davis (1980) and Williams and Smith (1974) further demonstrate a lack of consistency, reporting requirements of 0.65 and 0.25 g/d $BW^{0.73}$ respectively.

Estimating animal requirements for any nutrient is, by nature, difficult. AA are no exception as the full extent to which an AA is limiting cannot be measured unless all other nutrients are provided in excess of requirements; this is a difficult task when trying to maintain realistic experimental conditions (Zimmerman and Scott, 1965). Despite the broad range in predicted AA requirements, methionine and lysine have frequently been shown to be first limiting for growing cattle (Richardson and Hatfield, 1978; Oke et al., 1986; Klemesrud et al., 2000).

Given that corn is deficient in lysine (Shewry, 2007) and microbial protein provides limited quantities of methionine (Storm and Ørskov, 1984), it is likely these AA are limiting for growing cattle fed corn-based diets. The objective of this study was to determine which amino acids were limiting for cattle fed corn-based diets. To accomplish this, we evaluated the effects of removing specific AA from a post-ruminal infusion containing all essential AA on nitrogen retention, a measure of protein deposition.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved all procedures involving the use of animals (IACUC-4902).

Animals and Experimental Treatments

Six steers from Chapter 2 (BW at trial initiation = 238 ± 12 kg) were used in a subsequent experiment of similar design but with different treatments. Steers were allowed 5 d in tie-stalls following the Chapter 2 trial before being placed in metabolism crates where they received abomasal water infusions for 3 d prior to the start of the experiment. Daily feed was increased to 6.0 kg/d (as fed basis) upon completion of Chapter 2 trial. All treatments were infused abomasally and included: 1) 12 g/d lysine, 4 g/d methionine, 4 g/d histidine, 4 g/d leucine, 4 g/d isoleucine, 4 g/d threonine, 4 g/d arginine, 4 g/d phenylalanine, 4 g/d valine, and 1 g/d tryptophan (MIX); 2) MIX without lysine (–Lys); 3) MIX without methionine (–Met); 4) MIX without histidine (–His); 5) MIX without valine, leucine, and isoleucine (–BCAA); 6) MIX without threonine and tryptophan (–Trp/Thr).

A lysine treatment solution was prepared by dissolving 152.8 g lysine-HCl (120 g L-lysine) in 3,847.2 g water. A branched-chain solution was prepared by dissolving 40.0 g leucine, 40 g isoleucine, and 40 g valine into 1,080.0 g water. An arginine and phenylalanine solution was prepared by dissolving 48 g of arginine and 48 g of phenylalanine into 9,504 g water. A threonine and tryptophan solution was prepared by dissolving 40 g threonine and 10 g tryptophan in 7,950 g water. Lastly, a histidine solution was prepared by dissolving 54.0 g histidine-HCl-H_2O (40 g L-histidine) in 3,946 g water. The treatment solutions were refrigerated from mixing until use. Infusion bottles (2 per steer per day, each providing for 12 h of infusion) were prepared by adding treatment solutions and water to a total weight of 2 kg. These stock solutions were

added in fixed quantities to provide the targeted AA dosage, e.g., for steers receiving 12 g/d lysine, 200 g lysine solution was added to each bottle.

Steers were housed in the same conditions as Chapter 2 after a 5-d rest period in tie stalls between experiments. Daily feed allocation was 5.3 kg/d (dry matter basis). Treatment solutions were infused abomassally with total solution amounts of 4 kg/d.

Sample Collection

Feed ingredients were sampled individually prior to diet mixing and stored at –20 °C. Representative diet samples from every period were collected at each feeding on d 2 to 5 and stored at –20 °C. Diet samples were composited within period then subsampled and stored at –20 °C. Orts from d 2 to 5 were collected and weighed, composited by steer within each period, and then subsampled and stored at –20 °C. Urine and feces from d 3 to 6 were collected and weighed daily. Urine was collected in buckets containing 900 mL of 10% (wt/wt) H_2SO_4 for ammonia preservation. Feces were collected in pans lined with plastic bags to facilitate mixing. Representative samples of urine and feces were obtained from the daily samples and stored at –20 °C. Orts, feces, and urine were composited by steer within each period; composites were created as proportions of total outputs and stored at –20 °C.

Jugular blood samples were collected on d 6 of each period at 4 h after feeding into 7.5 mL sodium heparin tubes (SARSTEDT Inc., Newton, NC). Tubes were immediately inverted, placed on ice, and centrifuged at 1,200 × g at 4 °C for 20 min. The supernatant was transferred to a 2-mL microcentrifuge tube and stored at –20 °C.

Laboratory Analyses

Diet and orts samples were dried in a 55 °C forced-air oven for 48 h, allowed to air-equilibrate, weighed, and then ground through a 1-mm screen with a Thomas Wiley Mill

(Thomas Scientific, Swedesboro, NJ). Samples were dried in a 105 °C forced-air oven for 24 h, followed by 12 h at 450 °C in a muffle oven to determine total dry matter (DM) and organic matter (OM) content. Wet fecal samples were dried in a 105 °C forced-air oven for 24 h to determine total dry matter (DM), followed by 12 h in a 450 °C muffle oven to determine OM content. Nitrogen content was measured in duplicate for diet, orts, wet feces, and urine with a LECO Nitrogen Analyzer (LECO Corporation, Saint Joseph, MI).

Plasma samples (0.75 mL) were deproteinized prior to amino acid analysis by mixing plasma with an equal volume of 10% (wt/vol) sulfosalicylic acid containing 600 μM norvaline as an internal standard. Samples were vortexed and placed on ice for 1 h, with recurring agitation every 15 min. Samples were then centrifuged at 17,000 × *g* for 20 min at 4 °C, the supernatant was filtered through a 0.2-micron syringe filter (Thermo Fisher Scientific Inc., Waltham, MA), and then stored at –20 °C before thawing and derivatizing using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AccQ-Tag Ultra Derivatization Kit; Waters Corporation, Milford, MA). Derivatized samples were analyzed using ultra-high pressure liquid chromatography (Waters Acquity UPLC; Waters Corporation, Milford, MA). Samples were separated using a C18 column and detected with a spectrophotometer at 260 nm.

Calculations

Digestibilities of DM and OM were calculated with intake solely from dietary origin. Retained nitrogen was calculated as the difference between nitrogen intake (intake + infused) and nitrogen output (urinary + fecal).

Data and Statistical Analysis

The experiment was designed to provide six observations per treatment, but some observations were omitted due to large feed refusals, failed treatment infusion, or steer health-

related issues. Specifically, two observations were lost for –Lys and –His, and one observation for MIX, –Met, and –Trp/Thr. Data were analyzed as a Latin square with fixed effects of treatment and period and random effect of steer using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Treatment means were derived from LSMEANS statement and separated with pairwise t-tests. Significance and tendencies were established at $P \leq 0.05$ and $0.05 < P \leq 0.10$ respectively.

Results and Discussion

Nitrogen Retention

Deletion of a limiting AA from a post-ruminal essential mix should inherently reduce nitrogen retention because other AA cannot be used as effectively in its absence. A comparison of the extent of reduction of nitrogen retention for each deleted AA provides insight into which AA is most limiting because the extent of AA limitation is related to the degree to which they limit protein deposition. Nitrogen retention (Table 2.2) was numerically greatest for steers receiving MIX (42.2 g N/d). The largest decreases in nitrogen retention were a result of –Lys (35.1 g/d; P=0.11) or –Met (35.6 g/d; P=0.11), followed by –Trp/Thr (36.6 g/d; P=0.17). Nitrogen retention was not significantly altered by –His (40.5 g/d; P=0.72) or –BCAA (39.7 g/d; P=0.51). Greenwood and Titgemeyer (2000) performed a deletion of lysine from a 10 EAA mix supplemented to Holstein steers, and reported a similar decrease in retained nitrogen with lysine removal.

Often, changes in nitrogen retention are associated with changes in nitrogen excretion though urine and occasionally feces (Abe et al., 1999; Donahue et al., 1985; Zou et al., 2023). However, under the conditions of this study, no treatment effects were detectable for urinary (P=0.70) or fecal (P=0.83) nitrogen excretion. In part, the expected increase in urinary nitrogen in response to AA deletion was offset by the reductions in total AA supplied, which prevented the urinary nitrogen excretion from being altered. As an example, deletion of lysine decreased nitrogen in the infusion by 2.3 g/d while urinary nitrogen was numerically (P=0.14) increased 4.0 g/d when lysine was removed from MIX. Greenwood and Titgemeyer (2000) conducted a trial of similar design, reporting a decrease in nitrogen retention (P<0.09) in response to lysine deletion from an essential AA mix. Although urinary nitrogen excretion was not significantly

altered, the decrease in infused nitrogen (2.8 g/d) likely offset the numerically greater urinary nitrogen excretion (0.7 g/d), further suggesting reductions in AA supply can moderate changes in urinary nitrogen excretion. The lack of significant treatment effects on urinary nitrogen excretion likely reflects limitations associated with experimental variation. However, the data is suggestive that methionine and lysine are most-limiting under our experimental conditions, and that tryptophan and/or threonine may have been additionally limiting.

Intake and Digestibility

Digestibility of both dry matter (P=0.04) and organic matter (P=0.02) were less for –Met than MIX, deviating from expectations as the impact of methionine on digestibility has been shown to be minimal (Donahue et al., 1985; Hall et al., 1996; Campbell et al., 1996). These findings may be attributable to variability rather than a change in digestive efficiency in response to supplemental methionine.

Plasma AA Concentrations

Effects of Removing Single Amino Acids from an Essential Amino Acid Mix

Three of six treatments involved the removal of a single AA from the MIX treatment, which allowed a direct assessment of those AA deletions on plasma AA concentrations. When lysine was removed, plasma lysine concentration was numerically decreased (P=0.43) from 108.4 to 98.5 μM; although not statistically significant this demonstrates the same relationship observed in Chapter 2. Additionally, removal of lysine resulted in an increase in the plasma concentrations, at least numerically, of all other measured AA except alanine and proline. This contributed to numerically larger (P=0.64) total AA concentrations as Lys was removed and is likely attributable to a reduced utilization of these AA for protein.

Plasma methionine was lower (P=0.13) for –Met (28.0 μM) than MIX (31.8 μM). Although not statistically significant, this was expected because a decrease in post-ruminal AA supply has previously been correlated with decreased plasma concentrations of that AA (Löest et al., 2001; Li et al., 2022). Plasma concentration of multiple AA including leucine, isoleucine, phenylalanine, glutamate, and ornithine, were increased (P<0.05) by –Met, further supporting the concept that reduced supply of an AA that becomes limiting can result in greater plasma concentrations of others, presumably a result of reduced utilization for protein deposition.

Plasma histidine concentrations were significantly lower (P=0.001) for –His (45.4 μM) than MIX (65.1 μM). Additionally, –His resulted in increased plasma concentrations (P<0.05) of methionine, isoleucine, phenylalanine, and aspartate. Increases in plasma concentrations of methionine, isoleucine, and phenylalanine could suggest a minor decrease in use for protein deposition, but removal of histidine from MIX did not reduce nitrogen retention much.

Collectively, the changes in plasma AA concentrations in response to individual AA deletion from an essential AA mix highlight expected outcomes. First, post-ruminal supply of each AA was related to plasma concentration of the individually deleted AA. Second, post-ruminal supply of potentially limiting AA may be inversely related to plasma concentration of other AA.

Effects of Removing Multiple Amino Acids from an Essential Amino Acid Mix

Two treatments involved the simultaneous deletion of multiple AA from MIX. Increased plasma concentrations (P<0.05) of lysine, leucine, isoleucine, valine, phenylalanine, arginine, aspartate, glutamate, ornithine, and tryptophan were observed for –Trp/Thr. These increases might be attributable to shifts in AA utilization for protein deposition as previously discussed. However, the increase in plasma tryptophan concentration is an anomaly because it increased in

the face of decreasing supply. Removal of both tryptophan and threonine from the infusion mixture also numerically increased (P=0.45) plasma threonine concentration. An obvious physiological pathway to explain this phenomenon is lacking, but we speculate that the decreases in protein deposition associated with deletion of these AA may have played a role in elevating plasma concentrations of tryptophan and threonine. In addition, there may be regulatory activities associated with degradation of tryptophan and/or threonine that were impacted directly by their joint removal or indirectly by the effect of their removal on protein metabolism.

Increased plasma concentration (P<0.05) of lysine, histidine, tryptophan, phenylalanine, arginine, glutamate, glutamine and leucine were observed for –BCAA. Numerical increases (P<0.21) were also observed for isoleucine and valine. Unlike some of the other treatments where increases in plasma AA concentrations can be attributed to reductions in protein deposition, there were only small, insignificant changes (P=0.51) in nitrogen retention when the BCAA occurred. As previously mentioned, the majority of the plasma AA concentrations increased as expected; however, a significant increase in plasma leucine and numerical increases in plasma isoleucine and valine deviates from expectations. Both Greenwood and Titgemeyer (2000) and Löest et al. (2001) deleted BCAA from a mix of 10 essential AA and reported significant decreases in plasma concentrations of leucine, isoleucine, and valine. In both studies, they supplemented much greater quantities of BCAA to the steers receiving the EAA mix and baseline plasma concentrations of the BCAA were greater than in our experiment. Nonetheless, the reason for the contradiction remains unclear. Metabolism of the BCAA is unique in that oxidation is carried out extensively by peripheral tissues outside the liver and catabolism is largely regulated by substrate availability (Harper et al., 1984). It may be that a decrease in BCAA supply decreased the activity of the enzymatic machinery essential for catabolism of the

BCAA, thus contributing to an increase in plasma concentrations despite deletion from post-ruminal supply. As well, indirect effects mediated through increases in concentrations of a number of AA could alter degradation of the BCAA, although we can provide no mechanism to support this conjecture. The unexpected increases in the BCAA concentrations in response to their removal from the AA mix further emphasizes that caution should be taken when drawing conclusions from plasma AA concentrations, especially when supplies of multiple AA are manipulated together.

Conclusions

We observed numerically decreased nitrogen retention when lysine and methionine were removed from an abomasally supplemented essential AA mix. Lysine and methionine appeared to be the first-limiting AA for growing cattle fed our corn-based diet, and the extent of their limitation appeared to be similar. As well, tryptophan and/or threonine may have been additionally limiting. Additional research will be needed to more clearly identify limiting AA and quantify the extent of each limitation under various experimental conditions.

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Table 3.1. Diet composition

Item	% of DM
Ingredient	
Dry-rolled corn	55.4
Dried corn gluten feed	23.0
Prairie hay	16.1
Molasses	3.1
Limestone	2.0
Trace mineral salt[1]	0.4
Vitamin and mineral premix[2]	0.1
Composition	
DM, % as fed	87.9
OM	92.7
CP	10.5

[1]Composition (at minimum): 96.0% NaCl, 0.24% Mn, 0.24% Fe, 0.032% Zn, 0.026% Cu, 0.007% I, and 0.004% Co

[2]Provided (per kg diet DM): 1800 IU vitamin A, 200 IU vitamin D, 20 IU vitamin E, 26.40 mg Zn, 8.80 mg Cu, 8.80 mg Mn, and 0.18 mg Se

Table 3.2. Effects of amino acid deletion from a mixture of abomasally supplemented essential amino acids on nitrogen utilization of growing steers fed a corn-based diet

	Treatment							
		Amino acid deleted[1]						
Item[2]	MIX	Lys	Met	His	Thr/Trp	BCAA	SEM[3]	Trt *P*
n=	5	4	5	4	5	6		
DMI, kg/d	5.18	4.99	4.89	5.19	4.95	5.12	0.20	0.59
OMI, kg/d	4.81	4.64	4.55	4.81	4.60	4.75	0.18	0.61
DMD, g/g	0.734	0.760	0.692	0.736	0.744	0.740	0.023	0.06
OMD, g/g	0.753	0.775	0.708	0.748	0.760	0.755	0.022	0.04
Nitrogen, g/d								
Feed	90.7	87.8	86.2	90.9	86.9	89.6	3.2	0.59
Infused	7.3	5.0	6.9	6.2	6.7	6.0	-	-
Total intake	98.0	92.8	93.1	97.1	93.6	95.6	3.2	0.49
Fecal	35.2	32.8	36.0	34.0	33.8	33.4	2.7	0.83
Urine	20.4	24.4	21.5	22.1	23.1	22.5	2.5	0.70
Retained	42.2	35.1	35.6	40.5	36.6	39.7	3.7	0.42
Nitrogen, % of total intake								
Fecal	35.9	35.3	38.8	35.3	36.1	35.0	2.3	0.57
Urine	21.0	26.6	23.3	23.4	25.0	23.7	3.4	0.70
Retained	43.2	37.9	37.9	41.7	38.9	41.3	3.2	0.50

[1]MIX = 12 g/d lysine, 4 g/d methionine, 4 g/d histidine, 4 g/d leucine, 4 g/d isoleucine, 4 g/d valine, 4 g/d threonine, 4 g/d arginine, 4 g/d phenylalanine, 4 g/d valine, and 1 g/d tryptophan; BCAA = leucine, isoleucine, and valine; SEM: standard error of mean.
[2]DMI = dry matter intake; OMI = organic matter intake; DMD = dry matter digestibility; OMD = organic matter digestibility.
[3]SEM = standard error of mean

Table 3.3. Effects of amino acid deletion from a mixture of abomasally supplemented essential amino acids on plasma amino acid concentrations of growing steers fed a corn-based diet

	Treatment							
		Amino Acid Deleted						
Item	MIX	Lys	Met	His	Trp/Thr	BCAA	SEM	Trt *P*
n=	5	4	5	4	5	6		
Lysine	108.4	98.5	126.9	124.3	139.7[A]	133.5[a]	10.4	0.05
Methionine	31.8	32.2	28.0	38.6[A]	34.6	35.7[a]	1.8	0.01
Histidine	65.1	73.5	68.8	45.4[A]	61.6	78.7[A]	4.7	<0.001
Tryptophan	31.3	34.6[a]	33.9	30.3	36.7[A]	35.4[A]	1.4	0.30
Threonine	91.1	97.0	94.9	88.8	98.8	110.0[a]	10.5	0.02
Leucine	144.3	144.6	170.7[A]	157.1	165.9[A]	167.4[A]	7.2	0.04
Isoleucine	99.0	102.0	119.2[A]	119.0[A]	117.0[A]	109.4	5.6	0.04
Valine	205.6	214.2	223.6	206.3	231.3[A]	219.2	9.9	0.18
Phenylalanine	66.0	72.3	79.1[A]	77.0[A]	87.2[A]	80.0[A]	3.3	0.002
Arginine	93.9	104.6	107.7[a]	101.7	113.1[A]	111.4[A]	5.6	0.15
Alanine	174.9	166.3	161.0	161.4	173.8	185.4	9.8	0.32
Asparagine	30.3	30.8	32.1	32.5	32.3	35.7[a]	2.2	0.46
Aspartate	4.6	4.9	5.3	6.2[A]	6.5[A]	5.5	0.5	0.04
Glutamate	162.9	170.6	184.0[A]	160.8	185.7[A]	180.3[A]	7.9	0.04
Glutamine	276.0	290.9	294.7	291.6	296.4[a]	322.5[A]	9.7	0.02
Glycine	163.9	175.0	161.3	162.4	171.0	176.8[a]	6.6	0.27
Ornithine	56.4	57.5	69.9[A]	53.9	71.6[A]	65.6	4.7	0.04
Proline	68.8	68.1	68.9	64.0	70.3	73.2	3.8	0.50
Serine	64.3	70.8	65.9	65.6	69.9	71.2	3.5	0.48
Taurine	43.2	46.9	41.8	51.2[a]	51.4	43.9	3.9	0.24
Tyrosine	77.7	79.1	83.3	80.8	86.3	84.7	4.2	0.50
Total	2080.0	2129.8	2231.7	2145.7	2295.4[A]	2299.1[A]	70.8	0.09

MIX = 12 g/d lysine, 4 g/d methionine, 4 g/d histidine, 4 g/d leucine, 4 g/d isoleucine, 4 g/d valine, 4 g/d threonine, 4 g/d arginine, 4 g/d phenylalanine, 4 g/d valine, and 1 g/d tryptophan; BCAA = MIX with leucine, isoleucine, and valine deleted; SEM = standard error of mean.
[A]Means bearing superscript letter differ from MIX ($P \leq 0.05$).
[a]Means bearing superscript letter differ from MIX ($0.05 < P \leq 0.10$).