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Effect of ruminal ammonia supply on lysine utilization by growing steers[1]

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ABSTRACT: Six ruminally cannulated Holstein steers (202 ± 15 kg) were used to study the effects of ruminal ammonia loading on whole-body lysine (Lys) utilization. Steers were housed in metabolism crates and used in a 6 × 6 Latin square design. All steers received 2.52 kg DM/d of a diet (10.1% CP) containing 82% soybean hulls, 8% wheat straw, 5% cane molasses, and 5% vitamins and minerals, and 10 g/d of urea (considered to be part of the basal diet) was ruminally infused continuously to ensure adequate ruminal ammonia concentrations. All steers were ruminally infused continuously with 200 g/d of acetic acid, 200 g/d of propionic acid, and 50 g/d of butyric acid and abomasally infused with 300 g/d of glucose continuously to increase energy supply without increasing microbial protein supply. Steers were also abomasally infused continuously with an excess of all essential AA except Lys to ensure that Lys was the only limiting AA. Treatments were arranged as a 3 × 2 factorial with 3 levels of urea (0, 40, or 80 g/d) continuously infused ruminally to induce ammonia loading and 2 levels of Lys (0 or 6 g/d) continuously infused abomasally. Treatments did not affect fecal N output ($P = 0.37$). Lysine supplementation decreased ($P < 0.01$) urinary N excretion from 51.9 g/d to 44.3 g/d, increased ($P < 0.01$) retained N from 24.8 to 33.8 g/d, increased ($P < 0.01$) plasma Lys, and decreased ($P \leq 0.05$) plasma serine, tyrosine, valine, leucine, and phenylalanine. Lysine supplementation also tended ($P = 0.09$) to reduce plasma urea-N. Urea infusions linearly increased ($P = 0.05$) retained N (27.1, 29.3, and 31.5 g/d) and also linearly increased ($P < 0.01$) urinary N excretion (31.8, 48.1, and 64.4 g/d), urinary urea (21.9, 37.7, and 54.3 g/d), urinary ammonia (1.1, 1.4, and 1.9 g/d), and plasma urea (2.7, 4.0, and 5.1 mM), and linearly decreased plasma alanine ($P = 0.04$) and plasma glycine ($P < 0.01$). Assuming that retained protein is 6.25 × retained N and contains 6.4% Lys, the incremental efficiencies of infused Lys utilization were 51%, 59%, and 69% for steers receiving 0, 40, and 80 g/d of urea, respectively, indicating that ruminal ammonia loads may improve the efficiency of Lys utilization. This is supported by observed increases in whole body-protein deposition in response to ammonia loading of our steers that were, by design, Lys deficient.

Key Words: amino acid, cattle, growth, lysine, utilization

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INTRODUCTION

Lysine is the second-limiting AA in ruminal microbial protein for growth of cattle (Richardson and Hatfield, 1978), and it can be the first-limiting AA when corn-based diets are fed (Burris et al., 1976; Abe et al., 1997). Lysine is subject to hepatic catabolism even when it is supplied in deficient amounts (Ball et al., 2007), which may contribute to inefficient utilization of Lys for protein deposition.

When ammonia was provided to ruminants, increases in hepatic urea-N production exceeded the supplemental ammonia-N (Wilton et al., 1988), and AA-N was utilized for hepatic ammonia detoxification (Lobley et al., 1995). The use of AA-N to detoxify ammonia could decrease AA availability for productive functions. In contrast, Luo et al. (1995) observed that ammonia could supply both N atoms required for urea synthesis in ovine hepatocytes,

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suggesting that use of AA-N would not be required for ammonia detoxification. Lobley et al. (1996) also found no effects of a supplemental ammonia load on catabolism of Leu, suggesting that stimulation of urea synthesis via ammonia supplementation does not require increased AA catabolism (Milano et al., 2000).

When growing cattle were limited by Met, ammonia loading did not affect N retention (Awawdeh et al., 2004), but Awawdeh et al. (2005) reported significant improvements in N retention when steers limited by Leu were given increased ammonia loads. Thus, ammonia loading may have different effects when different AA are limiting. Under certain conditions, the feeding of high-protein diets and the associated ammonia absorption from the rumen may decrease catabolism of essential AA by hepatocytes, thereby increasing the efficiency of use of some AA.

Our objective was to determine the effects of ruminal ammonia loading on the efficiency of Lys utilization by growing steers for whole-body protein deposition. We hypothesized that an increased supply of ammonia would improve the efficiency of Lys utilization.

MATERIALS AND METHODS

Animals and Treatments

All animal procedures were approved by the Kansas State University Institutional Animal Care and Use Committee.

Seven ruminally cannulated Holstein steers (202 ± 15 kg) were used in a 6 × 6 Latin square design. The seventh steer was allocated to treatments in the same sequence as another steer within the Latin square. Observations were excluded when steers exhibited erratic feed intake, and the number of useful observations obtained for each treatment are provided in the data tables. Treatments were arranged in 3 × 2 factorial with 3 levels of ruminally infused urea (0, 40, and 80 g/d) and 2 levels of abomasally infused Lys (0 and 6 g/d).

Steers were housed in metabolism crates within a temperature-controlled room at 22°C. Infusion lines of flexible Tygon polyvinylchloride tubing (2 mm i.d.) were placed in the rumen and abomasum through the ruminal cannula. A perforated vial was attached to the end of the ruminal infusion lines to prevent direct infusion of VFA onto the ruminal wall. Rubber flanges (7-cm diameter) were attached to the end of the abomasal lines to anchor them in the abomasum. Ruminal and abomasal infusates (each containing 4 kg of solution) were continuously infused using a peristaltic pump (Model CP-78002–10; Cole-Parmer Instrument Company, Vernon Hills, IL).

All steers had ad libitum access to water and received the same diet (Table 1) at 2.52 kg DM/d in 2 equal portions at 12-h intervals. The experimental diet (along with a basal ruminal infusion of 10 g urea/d that all steers received and that was considered part of the basal diet) was formulated to provide adequate amounts of ruminally degradable protein but limited metabolizable AA (Campbell et al., 1997).

Table 1. Composition of experimental diet

Item	% of DM
Ingredient	
Pelleted soybean hulls	81.7
Wheat straw	8.1
Cane molasses	4.8
Calcium phosphate	2.07
Sodium bicarbonate	1.31
Calcium carbonate	1.09
Magnesium oxide	0.44
Trace mineral salt[1]	0.22
Vitamin premix[2]	0.14
Sulfur	0.11
Se premix[3]	0.011
Bovatec-91[4]	0.018
Nutrient	
Crude Protein	10.1
OM	89.2

[1]Composition > 95.5% NaCl, 0.24% Mn, 0.24% Fe, 0.05% Mg, 0.032% Cu, 0.032% Zn, 0.007% I, and 0.004% Co.

[2]Provided 5,300 IU vitamin A/kg diet DM, 3,593 IU vitamin D/kg diet DM, and 48 IU vitamin E/kg diet DM.

[3]Provided 0.065 mg Se/kg diet DM from sodium selenite.

[4]Supplied 36 mg lasalocid/kg diet DM.

All steers were continuously infused with 200 g of acetic acid/d, 200 g of propionic acid/d, and 50 g of butyric acid/d into the rumen and 300 g of glucose/d into the abomasum as energy sources for the animal; these infusions served as energy sources for the animal without increasing ruminal microbial protein production. The ruminal infusates were prepared by mixing solutions containing acetic acid, propionic acid, and butyric acid with solutions providing the basal 10 g/d of urea plus each urea treatment (0, 40, or 80 g/d). Water was added to bring the daily ruminal infusate weight to 4 kg.

All steers received continuous infusions of an AA-containing solution into the abomasum (Table 2). This solution provided Glu, Gly, and all essential AA except Lys to ensure that only Lys was limiting (Greenwood and Titgemeyer, 2000). Daily abomasal infusates (Table 2) were prepared by dissolving branched chain AA in 833 g of water containing 72 g of 6 *M* HCl. After L-Leu, L-Ile, and L-Val were dissolved, L-Met, L-His-H_2O-HCl, L-Thr, L-Phe, L-Arg-HCl, L-Trp, and Gly were added to the mixture. Once dissolved, 330 g of dextrose (providing 300 g of glucose) was added to the AA mixture. Glutamate (as monosodium glutamate) was dissolved separately in 448 g of water. The Lys-HCl, used to provide the Lys treatment, was

Table 2. Abomasal infusate mixture

Ingredient	Amount
	g/d
L-Lysine-HCl[1]	0 or 7.64
L-Methionine	10
L-Threonine	15
L-Histidine-HCl-H_2O[2]	8
L-Phenylalanine	20
L-Arginine-HCl[3]	15
L-Tryptophan[4]	5
L-Leucine	20
L-Isoleucine	15
L-Valine	15
Monosodium glutamate	150
Glycine	40
Dextrose[5]	330
	mg/d
Pyridoxine-HCl	10
Folic acid	10
Cyanocobalamin	0.1

[1]Feed grade (78.8%); provided 0 or 6 g L-Lys/d.

[2]Provided 5.9 g L-His/d.

[3]Provided 12.4 g L-Arg/d.

[4]Feed grade (98%); provided 4.9 g Trp/d.

[5]Provided 300 g glucose/d.

dissolved separately as a 10% (wt/wt) solution before being added to the abomasal infusion mixture. The 3 solutions (AA/dextrose mixture, Glu, and Lys) were mixed together in individual bottles and water was added to bring the daily abomasal infusate weight to 4 kg. Pyridoxine HCl (10 mg/d), folic acid (10 mg/d), and cyanocobalamin (0.1 mg/d) were dissolved and added to the abomasal infusate because steers under these experimental conditions may become deficient in at least one of those vitamins (Lambert et al., 2004).

Sample Collection and Analysis

Experimental periods lasted 6 d (2 d of adaptation and 4 d for total collection of feces and urine). Two days of adaptations were adequate because the aforementioned treatments do not alter ruminal fermentation (Moloney et al., 1998; Schroeder et al., 2006). Preceding the study, steers were adapted to the experimental diet and to similar infusions for a prior experiment.

Representative samples of the experimental diet were collected from d 2 to 5 of each period, stored at -20°C, and ground in a Wiley Mill through a 1-mm screen (Thomas Scientific, Swedesboro, NJ) before analysis. Orts, if any, were collected from d 3 to 6, combined, stored at -20°C, dried at 55°C in a forced-air oven, and ground through a 1-mm screen before analysis. Total urine and feces were collected daily from d 3 to 6 of each period and weighed to determine total output. Urine was collected daily in buckets containing 900 mL of 10% (wt/wt) H_2SO4 to prevent loss of ammonia. Representative samples of feces (10% of total) and urine (1% of total) from individual steers were pooled within period and stored at -20°C for later analyses.

Feed samples, orts, and feces were analyzed for DM in a forced-air oven for 24 h (105°C). These samples were subsequently ashed at 450°C for 8 h to determine OM. Feed samples, orts, wet feces, and urine were analyzed for N using a TruMac N Analyzer (LECO Corporation, St. Joseph, MI). Urinary urea was measured using the method of Marsh et al. (1965). Urinary ammonia was measured using the method of Broderick and Kang (1980).

Jugular blood samples were collected 10 h after the morning feeding on the last day of each period. Blood was collected into vacuum tubes (Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin. The tubes were immediately placed on ice, then centrifuged for 15 min at 1,000 × *g* at 4°C to obtain plasma. Plasma samples were stored at -20°C for later analysis of glucose, urea, and AA. Plasma glucose was measured using methods of Gochman and Schmitz (1972). Plasma urea was measured using the method of Marsh et al. (1965). Plasma samples were prepared for AA analysis and analyzed as described by Batista et al. (2015).

Statistical Analysis

Data were analyzed using the Mixed procedure of SAS System 9.3 for Windows (SAS Inst. Inc., Cary, NC). Fixed effects in the model included Lys, urea, Lys × urea, and period; steer was included as a random effect. Linear and quadratic effects of urea and their interactions with Lys were evaluated using orthogonal polynomial contrasts. Treatment means were calculated using the LSMEANS option. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

Efficiencies of Lys utilization were calculated by regressing Lys deposition (N retention, g N/d × 6.25 g protein/g N x 0.064 g Lys/g protein; Ainslie et al., 1993) against supplemental Lys within urea treatment using the Mixed procedure of SAS. The model included the random effect of steer, fixed effects of period and urea treatment, and Lys within urea treatment as a regression variable. Slopes were compared with contrasts that evaluated the linear and quadratic effects of urea level.

RESULTS AND DISCUSSION

Research Model

Greenwood and Titgemeyer (2000) determined that the experimental soybean hull-based diet used in

Table 3. Effects of abomasal Lys supplementation and ammonia loading via ruminal urea infusion on N balance and diet digestibilities in growing steers

	0 g/d of L-Lys			6 g/d of L-Lys								
	Urea, g/d							*P*-value[1]				
Item	0	40	80	0	40	80	SEM[2]	Lys	U-L	U-Q	Lys × U-L	Lys × U-Q
n	5	6	4	4	5	7						
Nitrogen, g/d												
Infused	39.4	58.0	76.5	40.6	59.1	77.7	–	–	–	–	–	–
Dietary	40.3	40.2	40.3	40.3	40.3	40.3	0.38	0.88	0.90	0.64	0.94	0.61
Total Intake	79.7	98.1	116.8	80.9	99.4	118.0	0.38	< 0.01	< 0.01	0.64	0.94	0.61
Fecal	21.7	21.5	21.3	20.7	21.5	21.7	1.3	0.75	0.72	0.78	0.37	0.84
Urinary	34.7	51.8	69.2	28.9	44.4	59.6	2.2	< 0.01	< 0.01	0.99	0.33	0.91
Ammonia	0.9	1.2	2.1	1.4	1.6	1.7	0.18	0.17	< 0.01	0.41	0.03	0.19
Urea	25.2	41.4	60.8	18.7	33.9	47.9	2.2	< 0.01	< 0.01	0.77	0.12	0.49
Retained	23.3	24.8	26.3	31.0	33.7	36.6	2.3	< 0.01	0.05	0.98	0.52	0.96
Digestibility, %												
DM	74.6	74.4	76.6	75.6	75.3	74.6	1.7	0.96	0.57	0.53	0.12	0.34
OM	76.2	76.1	78.3	76.9	77.0	76.2	1.6	0.84	0.44	0.63	0.14	0.30

[1]U-L = Linear effect of urea; U-Q = Quadratic effect of urea.

[2]For $n = 4$.

this study provided deficient amounts of Lys as well as of other essential AA, suggesting that this diet would be useful for studying Lys utilization in growing cattle. In our model, Lys was designed to be the sole limiting AA in growing cattle. The amount of intestinally absorbable Lys provided by our soybean-hull diet was 7.1 g/kg DMI (Campbell et al., 1997), so based on intakes of 2.52 kg DM/d, the experimental diet provided 17.9 g/d of absorbable Lys, which was not adequate for maximal N retention. By supplementing essential and nonessential AA (Table 2) above their suspected requirements, the confounding effects of other AA limiting protein deposition were avoided.

The 6 g/d Lys treatment was designed to be less than the upper range of the linear response surface for N retention, which was established by Batista et al. (2015) to be 9 g/d of supplemental Lys. This is important to ensure that responses to Lys supplementation in this study are within the linear response surface for N retention, which would allow estimates of Lys efficiency to be valid. If supplemental Lys amounts exceeded the steers' requirement, then efficiencies would be underestimated.

Another important aspect of the research model was that 10 g/d urea was ruminally infused to all animals regardless of treatments. This basal infusion of urea to all steers replaced the dietary urea that was provided in previous versions of our research model (Campbell et al., 1997). This modification was made to reduce variation in N intake that could come from imperfect mixing of supplemental urea with the diet. This urea was designed to provide ruminal ammonia concentrations necessary for optimal microbial growth and normal rumen function. The fact that diet digestibility and fecal N did not change with greater urea infusions would suggest that the basal ruminal infusion of urea was adequate to support microbial protein synthesis. Thus, our model likely eliminated ruminal fermentation as a confounding factor.

Nitrogen Retention

There were no significant interactions between Lys and urea for diet digestibility or N retention data (Table 3).

Effects of Lys. Abomasal Lys supplementation increased N intake ($P < 0.01$) from 98.2 to 99.4 g/d and increased ($P < 0.01$) N retention from 24.8 to 33.8 g/d. This increase was linked to decreased ($P < 0.01$) urinary N excretion from 51.9 to 44.3 g/d. Lysine infusion decreased ($P < 0.01$) urinary urea-N from 42.4 to 33.5 g/d, whereas urinary ammonia was not affected by Lys supply ($P = 0.17$). Lysine supplementation had no effect on fecal N ($P = 0.75$), DM digestibility ($P = 0.96$), or OM digestibility ($P = 0.84$).

The increases in N retention with Lys infusions indicated that steers were deficient in Lys in our research model, which was previously demonstrated by Batista et al. (2015). By making a limiting AA (Lys) available for protein synthesis, whole-body protein deposition (N retention) was enhanced and less AA were diverted toward catabolism. Such decreases in catabolism of AA other than Lys would appear as decreases in urinary N excretion, which was observed.

Effects of Urea. Nitrogen intake increased linearly ($P < 0.01$) with urea infusions, whereas fecal N output did not differ among treatments ($P = 0.37$). Between 0 and 80 g/d of urea infusion, total urinary N increased

linearly ($P < 0.01$) from 31.8 to 64.4 g/d, urinary urea-N increased linearly ($P < 0.01$) from 21.9 to 54.3 g/d, and urinary ammonia increased linearly ($P < 0.01$) from 1.1 to 1.9 g/d. With incremental urea infusions, N retention increased linearly ($P = 0.05$). At 0, 40, and 80 g/d of ruminally infused urea, steers retained 27.1, 29.3, and 31.5 g/d N, respectively.

The increase in N retention with urea infusions indicates that less Lys was catabolized by steers when greater ruminal ammonia loads were provided. There are a wide range of possible explanations for ways that ammonia might influence Lys utilization. Ammonia might affect Lys catabolism somewhat directly by changing substrate concentrations or by altering enzyme activity or amount. More general effects on protein deposition are also possible as a result of changes in endocrine regulation of synthesis or degradation of protein or in changes in blood flow to specific tissues such as the gut or liver. We did not evaluate mechanisms by which ammonia was acting, so any suggestions about mechanisms of action are speculative.

Lysine catabolism can occur in both the liver and small intestine (van Goudoever et al., 2000), so the Lys-sparing effect may have taken place in the liver, the small intestine, or at both sites. Previous work with pigs (Ball et al., 2007) indicated that the liver catabolizes a small proportion of Lys, even when the animal is fed below its Lys requirement. If this wasteful catabolism occurs in growing steers, the ruminal ammonia loads may have reduced hepatic Lys breakdown through some alteration of transport or metabolism. When faced with increased ammonia supply, the liver would increase ammonia detoxification, perhaps foregoing metabolism of less toxic compounds such as Lys.

van Goudoever et al. (2000) observed that 30% of whole-body Lys oxidation occurred in the small intestine when piglets were fed a high-protein diet. If this phenomenon is similar in cattle, the sparing of Lys by greater ammonia loads may have occurred in the intestine. This mechanism would presumably be dependent on some ruminal ammonia reaching the intestinal lumen and decreasing Lys catabolism by an unknown mechanism.

Efficiency of Lys Utilization

The efficiencies of Lys utilization were 51%, 59%, and 69% for steers receiving 0, 40, and 80 g/d of urea, respectively; neither linear ($P = 0.52$) nor quadratic ($P = 0.96$) effects of urea were significant for these efficiencies. Although the treatment differences were not significant, the numerical increases in incremental Lys efficiency were consistent with the overall increases in N retention in response to urea supplementation in our Lys-limiting model. Because the ammonia loading could affect utilization of the basal dietary Lys as well as the supplemental Lys, both overall N retention and incremental efficiency would be expected to move in the same direction; the similarity in response supports the conclusion that the efficiency of Lys utilization for protein deposition was increased by greater ruminal ammonia loads.

Batista et al. (2015) observed that the incremental efficiency of Lys utilization was 40%. In that study, experimental conditions were essentially equivalent to our lowest urea treatment, which yielded an incremental efficiency of 51%. The difference between studies is not extremely large and both values can be interpreted to suggest an inefficient utilization of Lys by growing cattle. Burris et al. (1976) also observed that Lys utilization was inefficiently used for protein deposition, and an efficiency of 13% for growing steers fed a corn-based diet can be calculated from that work. This lower value by Burris et al. (1976) may have resulted from the experimental conditions in that study. In the work of Burris et al. (1976), it is not certain that the cattle were limited by only Lys; if other AA were limiting, the response to supplemental Lys may have been incomplete.

Our calculated efficiency of Lys utilization at the lowest ammonia load (51%) was less than the NRC (1996) estimate of 65%, which is calculated based on BW of the cattle. Our average efficiency (60%) and the efficiency when 80 g/d of urea was provided (69%) were, however, reasonably close to the NRC (1996) prediction. Our measures of incremental efficiency of Lys utilization (51% to 69%) were substantially less than values near 100% that can be calculated from the data of Klemesrud et al. (2000a,b). One difference may be the use of ADG as the response criteria by Klemesrud et al. (2000a,b), whereas we based our calculations on N retention.

Urinary N Excretion

The increase in urinary urea with greater ruminal ammonia loads can be explained by greater urea cycle activity when more ammonia reached the liver for detoxification. The rise in urinary ammonia can be attributed to increased conversion of ammonia to Gln by perivenous hepatocytes that use ammonia as a substrate for amidation of Glu to Gln (Katz, 1992). Some of the Gln can be catabolized in the kidney to generate urinary ammonia. Our observed increases in urinary ammonia excretion in response to urea supplementation were consistent with those of McCuistion et al. (2004) when growing steers were limited by His and those of Awawdeh et al. (2005) when growing steers were limited by Leu. In the current study, as well as in the work of McCuistion et al. (2004) and Awawdeh et al. (2005), the increases in urinary ammonia were not large. In the current study, urinary ammonia represented only 3% of

the total urinary N, whereas urinary urea represented 84% of total urinary N at our highest level of urea infusion, suggesting that increases in total urinary N were mainly attributed to increases in urinary urea.

Urea infusions linearly increased (P = 0.04) total output of urine (acidified urine weights were 10.3. 11.0, and 12.3 kg/d for 0, 40, and 80 g/d urea, SEM = 1.2 kg/d; data not shown elsewhere). This increase may be attributed to the observed increases in urinary urea, which would increase urinary osmotic load and urinary water excretion. Lysine supplementation also increased (P = 0.03) total urine output (acidified urine weights were 10.3 and 12.0 kg/d for 0 and 6 g/d Lys, SEM = 1.1 kg/d; data not shown elsewhere). This response to Lys was unexpected because urinary urea excretion was decreased by Lys, which would decrease the urinary osmotic load. We used Lys-HCl as our Lys source, and the provision of Cl may have increased urinary excretion, although the amount of Cl was relatively small. Unknown factors, such as changes in hormonal secretion by the intestine in response to AA supply, also may have contributed to the response. Increases in urinary excretion in response to ammonia loading or Lys supplementation could have biological, environmental, and economic implications.

Implications of Ammonia Loading

The goal of ammonia loading in our study was to simulate conditions in which cattle consume high-protein diets, which consequently results in increased ammonia absorption from the gut. In a research setting, one can readily induce ammonia loading by feeding NPN sources such as urea. Once inside the rumen, urea is hydrolyzed by urease, predominantly absorbed as ammonia across the rumen epithelium, transported to the liver for detoxification as urea, and subsequently excreted in urine (Abdoun et al., 2006). The implications of this process on AA availability and protein deposition have been uncertain as prior research has yielded contradicting results. For example, Lobley et al. (1995) and Mutsvangwa et al. (1996) suggested that ammonia detoxification in hepatocytes contributed to inefficient use of AA. In those studies, AA extracted by the liver were presumably catabolized as a source of N to support urea synthesis. Conversely, Luo et al. (1995) and Milano et al. (2000) suggested that AA used for ureagenesis was not biologically important if acidotic conditions were avoided.

Previous studies in sheep (Norton et al., 1982) and cattle (Moorby and Theobald, 1999) found no changes in N balance when ammonia load was increased. For example, N balance remained constant when ammonium chloride was intraruminally infused into sheep fed a pelleted-grass diet (Norton et al., 1982). In Moorby and Theobald (1999), N balance changed numerically from 31 to 20 g/d in multiparous cows fed perennial ryegrass silage and duodenally infused with ammonium acetate. Although the authors reported no significant change, those experimental animals were not limiting in supply of an individual AA or of MP. For this reason, ammonia loading may have had limited observable effects on AA metabolism. The N retention responses in our study demonstrated that ammonia loading improved protein deposition in a model where Lys was designed to be limiting. The increases in N retention in response to urea infusion were consistent with McCuistion et al. (2004), who reported a numeric (P = 0.16) increase in N retention from 35.1 to 37.1 g/d when supplemental urea was increased from 0 to 80 g/d in growing steers limited by His. Awawdeh et al. (2005) observed significant improvements in N retention from 24.7 to 29.2 g/d when ammonia loading was induced in Leu-deficient steers. In contrast, Awawdeh et al. (2004) did not observe any changes in N retention of steers limited by Met when 40 and 80 g urea/d was ruminally infused. These findings suggest that differences exist among AA in how their utilization is affected by ammonia, although there is no clear pattern in their catabolic pathways that easily defines the differences. Lysine, Met, and His are primarily catabolized by the liver, whereas branch chain AA such as Leu are metabolized throughout the body. Additionally, the initial catabolic step for Leu is a reversible transamination reaction, whereas this is not the case for Lys or His. Taken as a whole, the results of studies with cattle limited by Met, His, Leu, or Lys support the concept that increased ammonia detoxification does not require AA-N inputs and, in the case of Lys and Leu, may reduce AA catabolism.

Plasma Metabolites

There was only 1 interaction (quadratic) between Lys and urea for plasma metabolites (Arg; Table 4) which had no clear explanation.

Effects of Lys. Lysine supplementation tended to decrease (P = 0.09) plasma urea (Table 4), but it had no effect on plasma glucose (P = 0.27). The numerical decreases in plasma urea with Lys supplementation were interpreted to suggest that less urea was produced from AA-N when a limiting AA was available for protein synthesis.

Lysine supplementation increased (P < 0.01) plasma Lys, Glu, and Arg concentrations and decreased (P < 0.05) plasma concentrations of Val, Leu, Ser, Tyr, Phe, and taurine (Table 4). Considering that steers in our model were Lys deficient, the increases in plasma Lys with supplementation of 6 g/d Lys would contradict the prediction of Bergen (1979) that plasma AA should not increase until the supply of that specific AA exceeds

Table 4. Effects of abomasal Lys supplementation and ammonia loading via ruminal urea infusion on plasma urea, glucose, and AA in growing steers

	0 g/d of L-Lys			6 g/d of L-Lys								
	Urea, g/d							*P*-value[1]				
Item	0	40	80	0	40	80	SEM[2]	Lys	U-L	U-Q	Lys × U-L	Lys × U-Q
n	5	6	4	4	5	7						
Urea, m*M*	2.9	4.5	5.1	2.4	3.4	5.1	0.63	0.09	< 0.01	0.80	0.54	0.27
Glucose, m*M*	5.9	6.4	6.3	6.3	6.5	6.3	0.33	0.45	0.60	0.27	0.56	0.86
Amino acid, μ*M*												
Lysine	21	23	22	39	37	38	5.4	< 0.01	0.97	0.93	0.81	0.60
Aminoadipate	1.70	1.81	2.06	2.55	2.06	2.00	0.31	0.10	0.71	0.50	0.11	0.73
Alanine	168	182	158	192	182	160	12.8	0.24	0.04	0.11	0.26	0.42
Arginine	65	74	67	79	71	87	7.9	< 0.01	0.33	0.61	0.47	0.02
Aspartic acid	10.5	13.0	9.6	13.8	13.4	11.9	1.7	0.12	0.39	0.19	0.77	0.35
Citrulline	73	69	76	66	65	71	7.5	0.16	0.35	0.27	0.87	0.76
Glutamate	74	77	77	102	90	88	7.4	< 0.01	0.39	0.75	0.23	0.56
Glutamine	289	315	286	294	285	280	29.4	0.52	0.65	0.45	0.78	0.39
Glycine	632	600	530	643	530	453	54.6	0.27	< 0.01	0.99	0.38	0.66
Isoleucine	66	67	70	65	68	64	6.3	0.68	0.83	0.76	0.66	0.57
Leucine	64	66	69	56	60	54	5.9	0.02	0.78	0.61	0.44	0.48
Methionine	34.7	33.5	32.2	31.3	34.5	31.2	3.9	0.61	0.65	0.50	0.68	0.53
Ornithine	54	55	56	61	53	50	8.7	0.93	0.56	0.86	0.37	0.85
Phenylalanine	73	70	71	68	69	58	3.9	0.05	0.12	0.49	0.24	0.18
Serine	120	127	109	94	95	80	7.2	< 0.01	0.06	0.07	0.77	0.69
Taurine	56	52	47	49	36	41	5.4	< 0.01	0.05	0.24	0.99	0.22
Threonine	157	148	154	153	168	135	19.5	0.94	0.47	0.50	0.64	0.21
Tryptophan	53	61	60	56	57	53	5.5	0.40	0.59	0.22	0.17	0.84
Tyrosine	50	54	53	38	40	44	6.4	< 0.01	0.38	0.84	0.84	0.70
Valine	258	272	274	243	235	226	21.1	0.02	0.99	0.83	0.32	0.87
Total AA	2,430	2,459	2,328	2,447	2,298	2,116	138.6	0.17	0.05	0.60	0.30	0.73

[1]U-L = Linear effect of urea; U-Q = Quadratic effect of urea.

[2]For *n* = 4.

the requirement for protein deposition, which Batista et al. (2015) determined to be 9 g/d for maximal N retention. However, Batista et al. (2015) reported increases in plasma Lys when 6 or 9 g/d of Lys was supplemented to steers under experimental conditions similar to our study. The increases in plasma Lys at supplementation amounts below the steers' requirement suggest that the prediction by Bergen (1979) is not applicable to plasma Lys in cattle. Although plasma Lys increased in response to Lys supplementation, it should also be noted that the Lys concentrations in plasma were not particularly high for any of the treatments.

Lysine supplementation tended ($P = 0.10$) to increase plasma concentrations of α-aminoadipate, an intermediate in the catabolism of lysine, and this matches observations of Batista et al. (2015). Also, the increases in plasma α-aminoadipate in response to lysine supplementation tended to be less when ammonia loading was created via urea infusion than when no urea was supplemented (Lys × urea-linear, $P = 0.11$). This observation would lead to the conclusion that ammonia loading decreased Lys catabolism, matching the observed improvements in the efficiency of Lys utilization in response to ammonia loading.

The observed decreases in plasma Val, Leu, Ser, Tyr, and Phe concentrations with Lys infusions may have resulted from increased uptake and use for protein deposition when Lys, the sole limiting AA in our study, was provided. If appropriate concentrations or amounts of each AA are not available to peripheral tissues, protein deposition can be limited. Decreases in plasma concentrations of Val and Leu in response to supplementation of a limiting AA were also observed in growing steers limited by Met (Awawdeh et al., 2004) and His (McCuistion et al., 2004). Additionally, Awawdeh et al. (2004) observed decreases in Tyr when Met-deficient steers were supplemented with Met. Decreases in plasma taurine in response to Lys supplementation might suggest that less taurine was produced as a product of Met metabolism subsequent to increased Met utilization for protein deposition (Stipanuk and Ueki, 2011).

It would be tempting to suggest that the increases ($P < 0.01$) in plasma Glu in response to Lys supplementation were a result of changes in ureagenesis that affected

Glu production or utilization; however, the lack of a plasma Glu response to urea infusion does not support this concept. The instability of Gln in plasma samples, which results in conversion of Gln to Glu, also makes interpretation of plasma Glu concentrations difficult, and it should be noted that concentrations of Gln plus Glu were not affected by treatments. The increases in plasma Arg with Lys infusion are also difficult to interpret. It may be possible that Lys supplementation altered Arg synthesis, Arg catabolism, or Arg excretion via changes in either enzymatically-mediated processes or in Arg transport.

Effects of Urea. Supplemental urea induced ammonia loading as demonstrated by linear increases ($P < 0.01$) in plasma urea (Table 4). The linear increase in plasma urea with urea infusions can be explained by increases in hepatic ureagenesis from ammonia absorbed across the rumen wall (Reynolds and Kristensen, 2008). Once released into venous blood, urea can either be excreted in urine by the kidney or be recycled to the gastrointestinal tract.

The lack of changes in plasma Lys with urea infusions was interpreted to suggest that there were offsetting effects on Lys concentrations. Improvements in Lys efficiency would reflect decreases in Lys catabolism, which could lead to increases in plasma Lys concentration. At the same time, the observed increases in protein deposition with greater ammonia loads could increase removal of Lys from plasma and potentially decrease its concentration. If both processes occurred at the same time, there might be little or no net change in Lys concentration.

Plasma Gly, Ala, taurine, and total AA linearly decreased ($P < 0.05$) and plasma Ser also tended to decrease linearly ($P = 0.06$) with urea infusions. Significant decreases in plasma Gly with urea infusions were consistent with McCuistion et al. (2004) when ammonia loading was induced in growing steers limited by His. McCuistion et al. (2004) attributed the decreases in Gly to increased utilization of Gly for synthesis of Asp, which can enter urea cycle when ammonia detoxification is upregulated in periportal hepatocytes. By using excess Gly as an Asp-N source for urea cycle, essential AA such as Lys may be spared from wasteful catabolism. This concept is supported by numerical decreases in Ser with greater urea infusions because Gly is converted to Ser by serine hydroxymethyltransferase (Fu et al., 2001); once produced, Ser can be deaminated to pyruvate and carboxylated to oxaloacetate, which is the direct precursor to Asp (Jitrapakdee and Wallace, 1999). Similar to plasma Gly, Ala concentrations were linearly decreased in response to ruminal ammonia loads, perhaps reflecting an increase in Ala utilization. Decreases in Ala were also reported when ammonia loading was induced in growing steers limited by Met (Awawdeh et al., 2004), His (McCuistion et al., 2004), and Leu (Awawdeh et al., 2005). Via transamination reactions, Ala may be used as a source of Asp-N for ammonia detoxification (Harper et al., 1984). Decreases in Ala in response to ammonia loading were consistent with an in vitro study by Mutsvangwa et al. (1996) where hepatocytes isolated from sheep supplemented with 20 g urea/kg DM had greater utilization of Ala for gluconeogenesis than hepatocytes from sheep that did not receive supplemental urea.

Conclusion

Overall, Lys supplementation improved whole-body protein deposition when growing steers were deficient in dietary Lys. No metabolic cost for protein deposition was demonstrated when ammonia loading was induced in Lys-deficient steers. Instead, daily N retention increased with greater urea infusions, suggesting that ruminal ammonia loading may improve the efficiency of Lys utilization. Despite potential improvements in cattle performance, the environmental and economic pitfalls may outweigh the benefits of providing excess N supplies.

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