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Efficiency of lysine utilization by growing steers[1,2]

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ABSTRACT: This study evaluated the efficiency of Lys utilization by growing steers. Five ruminally cannulated Holstein steers (165 ± 8 kg) housed in metabolism crates were used in a 6 × 6 Latin square design; data from a sixth steer was excluded due to erratic feed intake. All steers were limit fed (2.46 kg DM/d), twice daily, diets low in RUP (81% soybean hulls, 8% wheat straw, 6% cane molasses, and 5% vitamins and minerals). Treatments were 0, 3, 6, 9, 12, and 15 g/d of L-Lys continuously abomasally infused. To prevent AA other than Lys from limiting performance, a mixture providing all essential AA to excess was continuously abomasally infused. Additional continuous infusions included 10 g urea/d, 200 g acetic acid/d, 200 g propionic acid/d, and 50 g butyric acid/d to the rumen and 300 g glucose/d to the abomasum. These infusions provided adequate ruminal ammonia and increased energy supply without increasing microbial protein supply. Each 6-d period included 2 d for adaptation and 4 d for total fecal and urinary collections for measuring N balance. Blood was collected on d 6 (10 h after feeding). Diet OM digestibility was not altered ($P \geq 0.66$) by treatment and averaged 73.7%. Urinary N excretion was decreased from 32.3 to 24.3 g/d by increasing Lys supplementation to 9 g/d, with no further reduction when more than 9 g/d of Lys was supplied (linear and quadratic, $P < 0.01$). Changes in total urinary N excretion predominantly were due to changes in urinary urea N. Increasing Lys supply from 0 to 9 g/d increased N retention from 21.4 to 30.7 g/d, with no further increase beyond 9 g/d of Lys (linear and quadratic, $P < 0.01$). Break-point analysis estimated maximal N retention at 9 g/d supplemental Lys. Over the linear response surface of 0 to 9 g/d Lys, the efficiency of Lys utilization for protein deposition was 40%. Plasma urea N tended to be linearly decreased ($P = 0.06$) by Lys supplementation in agreement with the reduction in urinary urea N excretion. Plasma concentrations of Lys linearly increased ($P < 0.001$), but Leu, Ser, Val, and Tyr ($P \leq 0.02$) were linearly reduced by Lys supplementation, likely reflecting increased uptake for protein deposition. In our model, Lys supplementation promoted significant increases in N retention and was maximized at 9 g/d supplemental Lys with an efficiency of utilization of 40%.

Key words: amino acids, cattle, efficiency, lysine, nitrogen retention

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INTRODUCTION

The physiological requirements of cattle for AA must be considered to optimize performance, but microbial degradation of dietary protein and AA in the rumen creates a challenge for developing accurate estimates of requirements for ruminants (Titgemeyer, 2003).

Lysine was identified as the second-limiting AA when ruminal microbial protein was the main source of MP in growing cattle receiving semipurified diets (Richardson and Hatfield, 1978) as well as for sheep maintained by intragastric nutrition (Strom and Ørskov,

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1984). However, for corn-based diets, which are rich in Met supply, Lys was the first-limiting AA for growing cattle (Burris et al., 1976; Hill et al., 1980; Abe et al., 1997).

Estimates of the Lys requirements of growing steers have been developed using plasma data (Fenderson and Bergen, 1975), modeling approaches (O'Connor et al., 1993), and growth studies (Klemesrud et al., 2000a,b). The Cornell Net Carbohydrate and Protein System (O'Connor et al., 1993) bases efficiencies of AA utilization on relationships between absorbed and deposited protein, and equivalent shrunk BW is the sole factor used to estimate the efficiency of AA utilization. The same efficiency is applied to each essential AA as well as to MP. However, recent evaluations of Met (Campbell et al., 1996, 1997; Löest et al., 2002), Leu (Awawdeh et al., 2005, 2006; Titgemeyer et al., 2012), and His (McCuistion et al., 2004) utilization showed that differences exist among AA with regard to the efficiency of utilization. These inherent differences in the efficiency of individual AA utilization reflect differences in oxidation rates (Heger and Frydrych, 1989) and diverse metabolic processes for each AA (Owens and Pettigrew, 1989).

Our objective was to determine the efficiency of Lys utilization for whole-body protein deposition by growing steers.

MATERIALS AND METHODS

Animals and Treatments

The Kansas State University Institutional Animal Care and Use Committee approved procedures involving animals in this study.

Five ruminally cannulated Holstein steers (165 kg) were used in a 6 × 6, balanced Latin square design. A sixth steer was included in the experiment, but erratic feed intake by this animal led to exclusion of all data collected from it. Animals were housed in individual metabolism crates in a temperature-controlled room (20°C) under 16 h of light daily. Before the experiment, steers were adapted to the basal diet (Table 1) for 2 wk and to ruminal and abomasal infusions for 5 d. All steers had free access to water and received the same basal diet at 2.5 kg of DM/d in equal proportions at 12-h intervals. The diet used in this study (Table 1) was formulated to provide small amounts of metabolizable AA to create a limitation in Lys.

Treatments included daily abomasal infusions of 0, 3, 6, 9, 12, or 15 g of L-Lys/d. Treatments were continuously infused into the abomasum via peristaltic pump (model CP-78002-10; Cole-Parmer Instrument Company, Vernon Hills, IL) by placing Tygon tubing (2.0 mm i.d.) through the ruminal cannula and the reticulo-omasal orifice and into the abomasum. A rubber flange (10 cm diameter) was attached to the end of the abomasal infusion lines to ensure that they remained in place.

Table 1. Composition of the diet fed to growing steers

Item	Percent of DM
Ingredient	
Pelleted soybean hulls	81.7
Wheat straw	8.1
Cane molasses	4.8
Calcium phosphate (22% P)	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	
DM, %	89.3 ± 0.07
OM, % of DM	90.1 ± 0.17
N, % of DM	1.40 ± 0.02

[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 (Zoetis, Florham Park, NJ).

All steers received a basal infusion of a mixture providing daily 20 g of L-Leu, 15 g of L-Thr, 8 g of L-His·HCl·H_2O, 20 g of L-Phe, 5 g of L-Trp, 10 g of L-Met, 15 g of L-Ile, 15 g of L-Val, 15 g of L-Arg, 150 g of monosodium glutamate, and 40 g of Gly. The mixture was continuously infused into the abomasum to provide all essential AA except Lys in excess of the steers' requirements and prevent limitations in protein deposition by AA other than Lys. The profile of AA infused was based on the supplies and requirements of AA estimated for growing Holstein steers fed with a diet similar to that used in our study (Greenwood and Titgemeyer, 2000). Abomasal infusate for each steer was prepared by dissolving L-Leu, L-Ile, and L-Val in 1 kg of water containing 60 g of 6 *M* HCl. Once L-Leu, L-Ile, and L-Val were dissolved, the remaining AA, except Glu (monosodium glutamate), were added to the mixture. Monosodium glutamate was dissolved separately in 500 g of water. After all AA were dissolved, the 2 solutions of AA were mixed together, dextrose was added (330 g supplying 300 g glucose) to provide an additional energy source, and water was added to bring the total weight of the daily infusate to 4 kg. All steers received 10 mg of pyridoxine·HCl/d, 10 mg of folic acid/d, and 100 μg of cyanocobalamin/d in the abomasal infusate because the experimental diet was deficient in at least one of those vitamins (Lambert et al., 2004).

To ensure adequate ruminal ammonia concentrations to support microbial growth, all steers received a ruminal infusion of 10 g of urea/d; this was considered part of the dietary N intake. In addition, all steers received continuous ruminal infusions of 200 g of acetic acid/d, 200 g of propionic acid/d, and 50 g of butyric acid/d, with the total weight of the ruminal infusate being 4 kg/d. This supplemental energy was provided because dietary energy intake was restricted and energy deficiencies would limit the ability of the steers to retain N. Additionally, these energy sources can be utilized by the steers without affecting ruminal microbial protein synthesis. Infusion lines were placed in the rumen through the ruminal cannula and a perforated vial was attached to the end of the ruminal infusion lines to avoid direct infusion of VFA onto the ruminal wall.

Sample Collection and Analyses

Each experimental period lasted 6 d, with 2 d for adaptation to treatment and 4 d for total fecal and urinary collections. The 2-d adaptation periods were adequate because cattle rapidly adapt to changes in nutrients supplied postruminally (Moloney et al., 1998; Schroeder et al., 2006). Representative samples of the basal diet for each period were collected daily and stored (–20°C) for later analysis. Orts, if any, were collected on d 2 through 5, composited, and stored (–20°C) for later analysis. Feces and urine from each steer were collected from d 3 through 6 of each period and weighed to quantify total output. Urine was collected in buckets containing 900 mL of 10% wt/wt H_2SO_4 to prevent NH_3 loss. Representative samples of feces (10%) and urine (1%) were frozen daily, composited by period, and stored (–20°C) for later analysis. Before analysis, samples were thawed and homogenized. Feed and fecal samples were partly dried at 55°C for 36 h, air-equilibrated for 36 h, and ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ) through a 1-mm screen. Partly dried diet and fecal samples were analyzed for DM (105°C for 24 h) and ash (450°C for 8 h). Total N content was measured for feed, orts, wet fecal samples, and urine samples using a combustion analyzer (True Mac; Leco Corporation, St. Joseph, MI). Urine samples were analyzed colorimetrically for NH_3 (Broderick and Kang, 1980) and urea concentrations (Marsh et al., 1965).

On d 6 of each period, 10 h after the morning feeding, jugular blood was collected into vacuum tubes containing sodium heparin (Becton, Dickinson and Company, Franklin Lakes, NJ). Blood samples were immediately placed on ice and then centrifuged (1,000 × *g* for 20 min at 4°C) to obtain plasma, which was frozen (–20°C) for later analysis. Plasma urea and glucose concentrations were measured according to methods of Marsh et al. (1965) and Gochman and Schmitz (1972), respectively.

For AA analysis, plasma (750 μL) was mixed with 750 μL of a solution containing 4% sulfosalicylic acid, 0.3% lithium hydroxide, and 1.8% lithium chloride (SeraPrep; Pickering Laboratories, Mountain View, CA) containing 1 m*M* norleucine as an internal standard. After cooling on ice for 30 min, samples were vortexed and centrifuged (17,000 × *g* for 10 min at 4°C). The supernatant was used to measure plasma AA by cation exchange chromatography with postcolumn derivitization with *o*-phthalaldehyde. A 4 by 100 mm lithium ion-exchange column (Pickering Laboratories), a cation-exchange guard column (GARD Column; Pickering Laboratories), and lithium eluents (Pickering Laboratories) were used. Flow rate was 0.3 mL/min and the total run time was 130 min. The initial eluant (Li275) contained 0.7% lithium citrate, 0.6% lithium chloride, and 0.2% sulfolane and was pumped for 39.1 min. The second eluant (Li365) contained 2.7% lithium citrate and <0.1% lithium chloride and was pumped for 18 min. The third eluant (Li375) contained 3.4% lithium chloride and 1.5% lithium citrate and was pumped for 21 min. The fourth eluant (70% Li375:30% RG003) contained 2.56% lithium chloride, 1.05% lithium citrate, and 0.18% lithium hydroxide and was pumped for 30 min, after which the initial eluant was used to re-equilibrate the column for 21.9 min. The column temperature was 33°C for the initial 25 min and was then increased to 70°C for 85 min before being returned to 33°C for the final 20 min. The *o*-phthalaldehyde reagent, containing 0.3 g *o*-phthalaldehyde (Pickering Laboratories) dissolved in 3 mL methanol, 2 g *N*,*N*-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor; Pickering Laboratories), and 3 mL of 30% (wt/wt) Brij 35 (Ricca Chemical Co., Arlington, TX) dissolved in 950 mL of 5.4% potassium borate (OPA Diluent; Pickering Laboratories) was mixed with column effluent at a rate of 0.3 mL/min and allowed to react for 10 s at 21°C before fluorescence detection with excitation at 330 nm and emission at 465 nm (HP 1046A Fluorescence Detector; Agilent Technologies, Santa Clara, CA).

Statistical Analysis

In addition to exclusion of all data from a sixth steer due to erratic feed intake, 2 observations from other steers (0 and 15 g/d of Lys) were lost due to problems not related to treatment.

Nitrogen balance and plasma metabolite data were statistically analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The model contained the fixed effects of Lys and period, and steer was included as a random effect. Linear and quadratic

Table 2. Effects of Lys supplementation on diet digestion and N balance in growing steers

	Supplemental L-Lys, g/d							*P*-value	
Item	0	3	6	9	12	15	SEM[1]	Linear	Quadratic
n	4	5	5	5	5	4			
DMI,[2] kg/d	2.46	2.38	2.49	2.50	2.46	2.48	0.05	0.45	0.76
OM intake,[2] kg/d	2.22	2.14	2.25	2.25	2.21	2.23	0.05	0.51	0.77
N, g/d									
Infused[3]	36.1	36.7	37.3	37.9	38.4	39.0	–	–	–
Dietary intake[4]	38.8	38.3	39.2	39.4	39.1	39.2	0.52	0.28	0.75
Total intake	74.9	75.0	76.5	77.3	77.5	78.3	0.52	<0.001	0.75
Fecal excretion	21.0	22.1	22.9	22.2	22.2	22.0	0.82	0.15	<0.01
Urinary	32.3	29.1	26.6	24.3	25.5	25.8	1.6	<0.001	<0.001
Urea	21.6	19.1	16.1	14.8	15.1	14.2	2.0	<0.001	0.05
Ammonia	0.83	0.81	0.75	0.56	0.64	0.71	0.12	0.21	0.32
Retained	21.4	23.8	27.0	30.7	29.8	30.6	1.2	<0.001	<0.01
Diet apparent digestibility,[5] %									
DM	72.7	71.9	71.5	72.1	72.6	72.2	1.9	0.96	0.54
OM	74.2	73.4	73.5	73.4	74.0	73.6	1.9	0.86	0.66

[1]For $n = 4$.

[2]Amount provided by the diet (does not include amounts provided by infusions).

[3]Amount provided by AA.

[4]Dietary N plus N from ruminally infused urea.

[5]Based on dietary intake (infusions not considered).

effects of Lys were tested using single degree of freedom contrasts. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

Lysine requirements from the N retention and plasma Lys data were estimated from treatment means using a single slope, broken-line model (Robbins, 1986) and the NLIN procedure of SAS.

RESULTS AND DISCUSSION

Utilization of Lys for Protein Deposition

Intake, N balance, and apparent diet digestibility data are presented in Table 2. Lysine supplementation did not affect ($P \geq 0.45$) either DM or OM intake because steers were limit fed and orts were absent or small. Apparent digestibilities of DM and OM were not altered ($P \geq 0.54$) by treatment and averaged 72.2 and 73.7%, respectively. These digestibilities were consistent with those reported by Löest et al. (2002) and Campbell et al. (1996, 1997) for a similar diet. Steers receiving no supplemental Lys had less fecal N output than other steers (quadratic, $P < 0.01$); the difference, although significant, was neither large nor expected. Urinary N excretion was decreased from 32.3 to 24.3 g/d by increasing Lys supplementation to 9 g/d, with no further reduction when Lys supplementation exceeded 9 g/d (linear and quadratic, $P < 0.01$). Changes in total urinary N excretion were predominantly due to changes in urinary urea N, and urinary ammonia N was not affected by treatment ($P \geq 0.21$). Increasing Lys supply from 0 to 9 g/d increased N retention from 21.4 to 30.7 g/d, with no further increase beyond 9 g/d of Lys (linear and quadratic, $P < 0.01$). These N retention responses indicate that steers were deficient in Lys in our research model.

Break-point analysis (Fig. 1A) estimated maximal N retention starting at 9.0 g/d supplemental Lys. Assuming that 1) responses to supplemental Lys up to 9 g/d are linear, 2) protein deposition is 6.25 × N retention, and 3) deposited protein contains 6.4% Lys (Ainslie et al., 1993), the efficiency of Lys utilization between 0 and 9 g/d supplemental Lys was 40% (1.01 g retained N/g supplemental Lys × 6.25 g protein/g N × 0.064 g Lys/g protein).

This efficiency of Lys utilization is less than the efficiencies near 100% that can be calculated from the growth data of Klemesrud et al. (2000a,b). However, van Weerden and Huisman (1985) calculated an efficiency of 35% using N retention as a response variable in preruminant calves fed a milk replacer designed to be first limiting in Lys, and this represents an efficiency close to that from our study (40%). The Cornell Net Carbohydrate and Protein System as well as the NRC (1996) assume the same utilization efficiency value for all AA, and that efficiency is based solely on the equivalent shrunk BW of the animal. The efficiency of AA utilization for cattle in our study (165 kg BW) would be estimated by the NRC (1996) to be 65%; our lower efficiency is incongruent with this prediction. The overestimation by the NRC (1996) for efficiency of utilization of supplemental AA has been previously noted for Met (Campbell et al., 1996). McCuistion et al. (2004) observed an efficiency of supplemental His utilization (65%) that was greater than

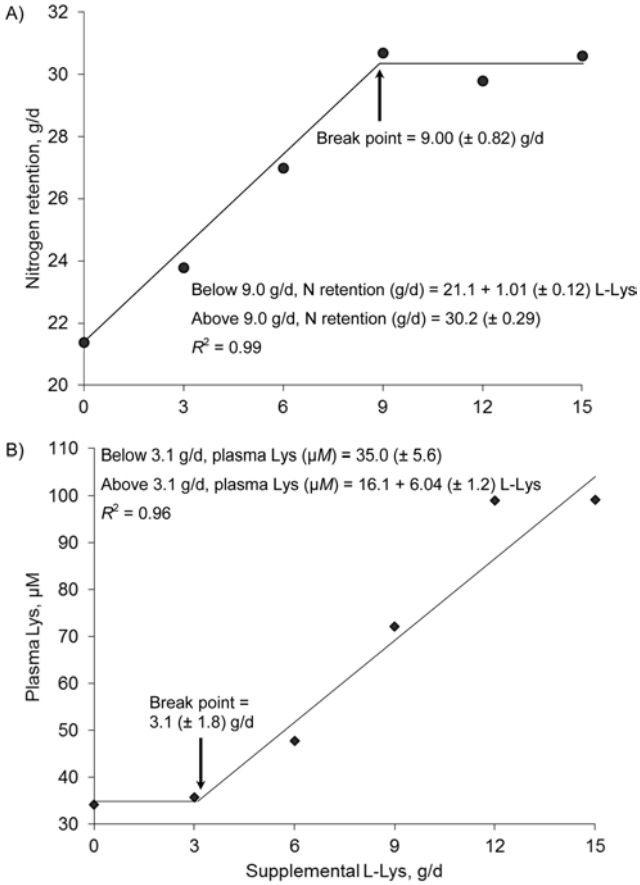

Figure 1. Nitrogen retention (A) and plasma Lys concentrations (B) of steers abomasally infused with L-Lys. Single-slope break-point analysis estimate of supplemental Lys requirement was 9.00 g/d based on N retention but only 3.12 g/d based on plasma Lys. The largest SEM for N retention means was 1.2 g N/d, and that for plasma Lys means was 8.1 µ*M*.

Table 3. Estimates of the efficiency of Lys deposition above maintenance for steers receiving no supplemental Lys

Item	Lysine[1]
Basal supply,[2] g/d	17.5
CP deposited,[3] g/d	133.8
Lysine deposited,[4] g/d	8.6
Gross efficiency,[5] %	49
Maintenance requirement,[6] g/d	9.9
Available for gain,[7] g/d	7.6
Efficiency above maintenance,[8] %	113

[1]Estimates for intestinally absorbed Lys supply to steers receiving no supplemental Lys. Supply = 2.46 kg DMI/d × 7.1 g Lys/kg DMI (Campbell et al., 1997).

[2]Based on measures of absorbable AA supplies for soybean hull–based diets (Campbell et al., 1997).

[3]Nitrogen retention (21.4 g/d) × 6.25.

[4]CP deposited × 0.064 (Lys concentration in tissue protein; Ainslie et al., 1993).

[5](Lys deposited/Lys available) × 100%, in which Lys available = basal supply.

[6]Represents the sum of scurf (0.032 × 0.2 × BW0.6/0.40), endogenous urine (0.067 × 2.75 × BW0.5/0.40), and metabolic fecal (0.064 × 0.09 × g/d of indigestible DM) requirements for 165-kg steers (O'Connor et al., 1993), in which 0.032 and 0.064 represent the concentration of Lys in keratin and tissue proteins, respectively. Indigestible DM was based on observed fecal DM output.

[7]Difference between basal supplies and maintenance requirements.

[8](Lys deposited/Lys available) × 100%, in which Lys available = Lys available for gain. This estimate of 113% is not feasible for an essential AA and it likely reflects an overestimation of the maintenance requirement, an overestimation of Lys deposition based on N retention, or both.

observations for Leu (approximately 41%; Awawdeh et al., 2006; Titgemeyer et al., 2012) but close to the NRC (1996) estimate for His utilization. The divergent estimated efficiencies among AA suggest that there are differences among AA in how efficiently they are used by cattle.

Our basal diet provided 7.1 g of intestinally absorbable Lys/kg DMI (Campbell et al., 1997), so based on intakes of 2.46 kg DM/d (Table 2), the basal supplies of absorbable Lys were 17.5 g/d. Steers receiving no supplemental Lys deposited 8.6 g Lys/d, which, assuming that maintenance requirements are 0, yields an efficiency of utilization of the basal Lys supplies of 49% (Table 3). If the *x*-axis in Fig. 1A is converted to total Lys supply by adding the basal supply of 17.5 g Lys/d to the supplemental amounts on the current *x*-axis, the regression line for N retention can be extrapolated to N retention of 0 to estimate the maintenance Lys requirements of the steers (assuming efficiency remains constant). This extrapolation yields a negative estimate for the maintenance requirement for Lys (–3.4 g/d), which is infeasible for an essential AA. The observation that maintenance requirements could be calculated to be negative may be explained by overestimation of protein deposition by N retention, efficiencies of utilization of the basal Lys that are greater than the efficiencies of utilization of the supplemental Lys, or both. Comparisons of N balance and serial comparative slaughter techniques have demonstrated that N retention generally overestimates protein deposition for growing cattle by 10 to 17% (Gerrits et al., 1996).

The Lys maintenance requirement for our steers, based on equations of O'Connor et al. (1993), was 9.9 g/d (Table 3). Using this estimate, 7.6 g Lys/d (basal supplies of 17.5 g/d minus 9.9 g/d for maintenance) would be available for growth when no supplemental Lys was provided, which is inconsistent with our estimate that these steers were depositing 8.6 g Lys/d (Table 3); estimates of efficiency of utilization above 100% are not feasible for an essential AA. Although protein deposition was calculated directly from N retention, N retention measurements would include scurf losses as part of retained N. As such, the scurf loss should be subtracted from N retention before using the relationship between N retention and Lys supply to estimate maintenance requirements. In practice, this correction will have little effect because scurf losses represent less than 4% of the estimated requirement.

Table 4. Effects of Lys supplementation on plasma metabolite concentrations of growing steers

	Supplemental L-Lys, g/d							*P*-value	
Item	0	3	6	9	12	15	SEM[1]	Linear	Quadratic
n	4	5	5	5	5	4			
Plasma									
Glucose, mM	5.59	5.64	5.62	5.74	5.78	5.36	0.22	0.74	0.26
Urea, mM	2.00	2.17	1.49	1.57	2.07	1.47	0.21	0.06	0.55
AA, μM									
Lys	34.2	35.7	47.8	72.1	99.0	99.2	8.1	<0.001	0.44
α-Aminoadipic acid	2.32	2.53	1.82	3.04	4.08	3.02	0.53	0.02	0.86
Ala	197.8	204.3	223.6	221.6	208.6	226.3	12.5	0.06	0.34
Arg	83.7	83.8	93.8	102.7	111.4	95.8	10.3	0.08	0.32
Asn	37.7	35.6	32.1	30.1	32.2	31.6	3.3	0.06	0.18
Asp	13.8	13.5	12.3	15.1	13.7	14.4	1.4	0.52	0.64
Citrulline	71.4	70.7	68.5	71.5	74.5	71.9	10.2	0.64	0.75
Glu	99.2	85.2	95.8	102.0	103.8	112.1	7.9	0.04	0.21
Gln	307.5	328.9	327.5	312.6	309.2	325.5	27.3	0.93	0.87
Gly	668.7	699.3	653.8	671.3	638.8	650.0	60.2	0.56	0.92
His	86.0	91.1	80.1	82.8	79.5	79.2	5.8	0.15	0.94
Ile	93.7	78.4	84.0	82.2	83.4	77.6	6.9	0.26	0.62
Leu	92.9	70.9	74.5	69.7	72.9	64.0	6.1	0.01	0.24
Met	47.3	42.0	45.2	44.2	40.7	42.3	5.7	0.51	0.87
Ornithine	63.3	60.1	69.8	77.9	84.2	70.1	12.4	0.17	0.41
Phe	99.4	83.9	91.3	84.3	89.7	75.9	6.5	0.07	0.99
Ser	137.4	122.4	100.3	88.1	92.3	91.9	8.7	<0.001	<0.01
Taurine	52.1	41.9	44.8	49.4	43.1	41.1	4.4	0.14	0.88
Thr	163.3	184.0	167.4	159.4	162.2	159.1	19.1	0.42	0.72
Trp	86.1	75.8	79.6	75.4	82.4	68.2	7.4	0.21	0.91
Tyr	91.1	79.1	65.7	61.2	64.5	60.2	6.9	<0.001	0.04
Val	330.6	285.3	287.2	274.5	279.5	258.3	17.5	0.02	0.38
Total AA	2,854	2,775	2,747	2,750	2,770	2,718	176	0.60	0.81

[1]For $n = 4$.

Using an approach similar to our study, Löest et al. (2001) observed that the efficiency of utilization of the basal supplies of Leu was greater than 100% when estimates for maintenance requirements were based on O'Connor et al. (1993). Similar calculations based on data of Campbell et al. (1997) for Met and of McCuistion et al. (2004) for His yield similar conclusions. Taken as a whole, the calculations discussed here and presented in Table 3 can be interpreted to suggest that for Lys, as well as for Leu, Met, and His, the maintenance requirements of growing cattle are less than estimated by O'Connor et al. (1993). The observation that the efficiency of utilization of the basal Lys was greater than 100% may be explained by some combination of 1) overestimation of maintenance requirements by O'Connor et al. (1993), 2) overestimation of protein deposition by N retention, and 3) efficiencies of utilization of the basal Lys that are greater than the utilization of the supplemental Lys (i.e., the efficiency of utilization above maintenance may not be a constant, although constant efficiencies have been observed for supplemental Lys [this study], Met [Campbell et al., 1997], and Leu [Awawdeh et al., 2005]).

Plasma Metabolites and AA

Effects of supplemental Lys on plasma metabolites and AA are shown in Table 4. Plasma urea N tended to be linearly decreased ($P = 0.06$) by Lys supplementation in agreement with the reduction in urinary urea N excretion, but plasma glucose was unaffected ($P \geq 0.26$) by Lys supplementation.

Plasma AA profiles are affected by different factors, which make it difficult to interpret the net result. Usually, an increase in plasma concentration of any essential AA in response to its supplementation would signify that the supply exceeds protein synthetic capacity as dictated by the first-limiting AA (Bergen, 1979). In an opposite way, a decrease in plasma concentrations of other AA, when a first-limiting AA is provided, would imply greater utilization for anabolic purposes (Gibb et al., 1992), because supplementation of the limiting AA should eliminate previous restrictions that the basal diet may have imposed on protein synthesis (Wessels et al., 1997).

Plasma Lys concentrations linearly increased ($P < 0.001$) with Lys supplementation. Plasma Lys remained low when 3 g/d Lys was infused and then

increased with greater levels of Lys supplementation. Break-point analysis of plasma Lys concentrations (Fig. 1B) estimated the supplemental Lys requirement of these steers to be 3.12 g/d, considering that levels of an AA will increase in plasma once the requirements for that AA have been exceeded (Bergen, 1979). The difference between requirements estimated from N retention data and plasma Lys data suggest that plasma Lys may be a poor indicator of requirements because its plasma concentration increased at supplementation levels that were clearly less than the amount needed to maximize protein deposition. This might suggest that increases in plasma Lys are associated with the mechanisms necessary to increase protein deposition in cattle, and this linkage of the protein deposition response to elevated concentrations of Lys may be a key to explaining why cattle utilize AA relatively inefficiently; elevated concentrations of AA would presumably increase their oxidation at the same time that they are needed to support greater protein deposition.

Plasma α-aminoadipic acid linearly increased ($P = 0.02$) in response to Lys supplementation. Mammalian catabolism of Lys occurs from 2 different routes: the pipecolate pathway predominates in the adult brain, whereas the saccharopine pathway is the predominant Lys degradative pathway in extracerebral tissues (Hallen et al., 2013). The main pathway yields α-aminoadipic acid as one of the intermediates (Voet et al., 2013); therefore, the increase in plasma α-aminoadipic acid probably reflects an increase in Lys catabolism as more Lys was supplied. Even when Lys was deficient, 60% of the supplemental Lys was not used for protein deposition (i.e., the efficiency of utilization was 40%), such that production of α-aminoadipic acid would be expected to increase with all levels of Lys supplementation.

Lysine supplementation linearly decreased plasma concentrations of Leu, Ser, Tyr, and Val ($P \leq 0.02$) and tended ($P \leq 0.07$) to linearly decrease Asn and Phe, probably reflecting the increased uptake and utilization of these AA for protein deposition. Previous studies also observed that supplementation with the most limiting AA decreases plasma concentrations of some of the other AA as protein retention is increased (Campbell et al., 1997; Wessels et al., 1997; Schroeder et al., 2006). Regarding Leu, Ser, and Val, decreases in plasma concentrations of these AA in response to supplementation of a limiting AA were also observed in growing steers limited by Met (Campbell et al., 1996; Awawdeh et al., 2004) or His (McCuistion et al., 2004).

On the other hand, plasma concentrations of Glu were linearly increased ($P = 0.04$) and Ala and Arg tended ($P \leq 0.08$) to linearly increase in response to Lys supplementation. The higher concentrations of Ala with levels of Lys possibly reflect decreases in Ala oxidation or increases in Ala synthesis via transamination reactions. Similarly, the increases in Glu might reflect less utilization of Glu for urea production because Lys supplementation decreased urea production, although increases in synthesis of Glu cannot be excluded as a possible explanation. Lysine and Arg are absorbed in the small intestine and kidney by the same transporters (Closs et al., 2004), and therefore, greater supplies of Lys could theoretically compete with Arg for transport. However, Abe et al. (1998) observed that antagonism between Lys and Arg did not occur in calves receiving excess Lys, demonstrated by increases in plasma Arg concentrations; in this regard, our study is in agreement with Abe et al. (1998).

Conclusions

Lysine supplementation promoted significant increases in N retention, demonstrating that our research model was limiting in this AA. The efficiency of utilization of supplemental Lys for whole-body protein deposition was 40%, which suggests that the efficiency of Lys utilization by growing cattle is somewhat less than would be predicted by the NRC (1996).

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